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Message from Editor-in-Chief

Uldis N. Streips, Ph.D.
Editor-in-Chief

It is a great pleasure to present this special issue of JIAMSE on the topic of Student Research projects. This issue looks at the value of the research that the two medical schools include in the medical curriculum toward an establishment of curiosity in the minds of medical students and future physicians. The abstracts indicate what the students are doing, while the articles provide a blueprint that medical schools around the world may choose to follow as curricula are revised. Enjoy.

Uldis N. Streips, Ph.D.
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Scientific Training in the Leiden Medical School Preclinical Curriculum to Prepare Students for their Research Projects

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ABSTRACT

In the undergraduate curriculum of Leiden Medical School, special attention is paid to the scientific training of all students. A four year transmodular program is offered which is fully integrated into the medical curriculum. The aspect of critical appraisal of scientific reports is a core element of the training, as well as development of several scientific skills needed for participation in research projects. The program aims to improve the skills of all students and to encourage those students with over-average academic ambitions to develop their skills further by voluntary courses and participation in high level medical research projects. The program is considered to be successful as a relatively high number of the Leiden medical students publish in scientific journals even before graduation.

INTRODUCTION

New knowledge in the field of medicine revises our understanding of the field with an increasingly rapid rate. It is important for physicians to be able to keep up-to-date with advancements and new developments in their fields of expertise. In general these new developments are published in the almost unlimited number of scientific research journals. For this reason, the AAMC-HHMI Committee recently indicated that students in medicine have to be supported to build a strong scientific foundation for future medical practice and have to be equipped with knowledge, skills and habits of mind to integrate new scientific discovery into their medical practice throughout their professional lives and to share this knowledge with patients and other health care professionals.[1]

Medical information from many other sources in addition to scientific journals comes across a doctor's desk, including advertisements and promotions from pharmaceutical companies. In many of these, research findings are reported with fancy graphs and references to support and add credibility to the message. However, it has been shown that many claims in such advertisements are not sufficiently evidence-based when the cited references are verified. [2] It is therefore essential that physicians also learn to judge the information presented and to separate "the wheat from the chaff". To this end, they must be able to read and critically appraise the many scientific papers that are published, which requires a specific additional set of skills.

All medical schools aim to deliver good physicians. Scientific training and research opportunities are often provided as part of the curriculum. At the Leiden University Medical Center (LUMC) scientific research training is provided for all undergraduate students, where the aspect of critical appraisal of scientific reports is a core element.

In addition, those students with over-average academic ambitions are encouraged to develop their skills even more by voluntary courses and participation in high level

medical research projects. In this paper, we describe the function and nature of this scientific training for medical students and discuss some of its outcomes.

THE LEIDEN MEDICAL SCHOOL CURRICULUM

Leiden Medical School is one of the eight state based medical schools in the Netherlands. Students enter Medical School after finishing secondary school education at an age of approximately 18 years old. Entry is mainly a centralized national process based on a numerus fixus system in which the government determines the number of first year students allowed to enter. Currently for Leiden Medical School this annual number is 325 first-year students. Undergraduate medical education consists typically of a 3-year Bachelor and a 3-year Master phase. There is no national exit exam and approximately 90% of all students entering Medical School finally graduate.[3]

The Leiden Medical School curriculum comprises a 3.5-year preclinical bachelor phase that offers general theoretical training, followed by a 2.5-year clinical master phase, consisting of internships at the LUMC or one of the associated general hospitals in the region. The preclinical, theoretical phase is patient-oriented and case-based: medical theory is always presented in relationship to real patient cases. The first two years focus on physiology and pathophysiology, whereas the third and fourth year are based on diseases, reproduction and aging using the Clinical Presentations model as developed in Calgary. [4] The entire preclinical phase is structured into multidisciplinary modules of 3 or 6 weeks. Educational formats used include: large group lectures and small group tutorials, interactive seminars, self-study activities, e-learning and early patient encounters. Typical small group size is 14 students. Each module is concluded with an individual paper-based exam that must be passed. Some subjects are not presented in modules, but in transmodular courses that are integrated with the modules and run throughout the year. These courses are concluded by an exam or by means of continuous assessment.

In the clinical phase students are divided into small groups of 14 students. Every two weeks one of these groups starts its 2.5 year of training. The first 1.5 years are spent in clinical clerkships of 2 to 10 weeks. In these clerkships the students are trained on the spot and experience an increasing level of responsibility in medical practice. During this period of clerkships 9 main disciplines are visited. All the students spend the last year of the clinical phase on scientific research in one or more research projects in the LUMC or another medical center in the Netherlands or abroad. Students can also choose to perform the scientific research projects just before entering the clinical clerkships, depending on availability and personal preferences.

SCIENTIFIC TRAINING IN THE PRECLINICAL PHASE

The scientific training program of Leiden Medical School consists of several modules offered in the preclinical phase of the curriculum.

Courses in year 1

During the first year of Medical School, the basics of epidemiology and biostatistics are discussed in the three-week course in public health. However, the main scientific training activity in this year is the transmodular course-Communication in Science (CiS). During this course all first-year students attend several lectures and participate in tutorials, during which they learn how to structure a scientific article and apply the standards of the scientific community. The basic principles of referencing and avoiding plagiarism are practiced, as well as rewriting strategies. Student pairs write a review article in Dutch and discuss their writing and that of other students by means of peer review, whereby the tutor acts as moderator. The CiS teaching concept is based on theories from sociolinguistics and discourse analysis. The main objective is for students to understand the function of language within their specific language community and how to apply language in the most effective way in professional interactive settings. In order to communicate effectively, students must further be aware of how they can amplify their strengths and diminish their weaknesses in these settings. Key topics such as audience interaction, the use of specific formats for scientific writing and presenting, the meaning of conventions, writing and speaking from the audience perspective and acknowledgment of sources, are taught and extensive personal feedback is provided. The original CiS program was designed in 1984. Since then, the CiS program has evolved into an integrated, transmodular course in written and oral scientific presentation skills in Dutch and English.

Courses in year 2

In the second year, students follow a three-week course in scientific training. The course focuses on several specific scientific skills and forms the foundation for scientific thinking and acting. It offers training in different study-designs and the basics of statistical data analysis. Also an integrated presentation skills course is offered on presenting the results of a research study in group meetings to colleagues. In the training, the conventions, rules and principles underlying an effective presentation in an academic setting are introduced and individual (video) feedback on a student's own performance is provided. Finally the ideas of the great philosophers, such as Plato, Hume and Popper are presented and discussed in six large group lectures.

During the course the students perform an individual assignment. First, in a small group setting the students discuss and judge a peer-reviewed article. During this

meeting key issues concerning the reliability of randomized controlled trials (RCTs), such as randomization, treatment allocation and blinding are discussed. After the meeting, the students receive two original RCTs from peer-reviewed journals, on which claims in medical advertisements are based. For this assignment the students use the JAMA journal guidelines.[5] In this way, during the course one hundred and fifty RCTs are critically examined and judged on their reliability and correctness in relation to the advertisement for which they are used. Following their analysis, each year a small group of students continue these investigations. The conclusion of the investigation as performed in 2005 was that claims can be easily misleading, and thus advertisements should be critically appraised**.** This conclusion was written by a few students and published.[2] Results of the investigations of recent years are currently in preparation for publication.

Courses in year 3

Evidence-based medicine is the core of a three-week course on Clinical Medicine at the beginning of the more clinically oriented third year of the curriculum. Starting with a case of a patient presenting with pain in the chest, all steps in clinical diagnostic reasoning are made explicit. The concept of prior probability of disease is explained in a Bayesian context, and students learn how to estimate posterior probability conditional on a specific test result using sensitivity and specificity of a test reported in the literature. Students apply these skills in critical appraisal of a paper on the value of spiral CT in making the diagnosis of pulmonary embolism. For choosing a proper treatment, a decision tree is explained. Instead of a normal written exam, each student gets a written description of either a male or a female patient with a certain age, specific combination of a medical history, clinical symptoms, and laboratory values. All students have to answer the same questions about their own patient in a take-home exam on which they spent 10-15 hours. Students are allowed to discuss and work together with other students, but at the end of the day they have to send in all answers to the questions concerning their own patient. Questions are related to constructing a differential diagnosis for their patient, designing a diagnostic strategy, and finding data on sensitivity and specificity of these tests in the literature. The exam contains also a few calculations on 2x2 tables, where the numbers in the tables vary between students. As a final exercise in this examination, students have to write a half page letter to their patient in response to his or her question why the proposed diagnostic strategy is better than a fictive test the patient had found on the internet. This part of the exercise tests the active understanding of the student in a semi-real life situation.

In the other clinical courses during the third year, students spend several hours studying and discussing a more scientific approach to clinical problems. In each course, a lecture is given to stimulate reflection and scientific reasoning. For example, in the course on clinical nephrology, the nephrologists explain the importance of early recognition and treatment of chronic renal failure. In a special lecture, the evidence for the effectiveness of screening programs in this field is discussed in the context of the Wilson and Jungner landmark paper on criteria for applying screening programs.[6] Then students are challenged to think about the gap between what seems to be reasonable from both a pathophysiological or clinical point of view, versus the effectiveness on a wider scale.

Courses in year 4

A three-week course on practical research skills is provided in the fourth year as a final preparation for the individual research project. In this course, specific attention is paid to formulating a scientific research question and choosing a proper study design. All students read two papers and write a study protocol to advance in this line of research. In addition, they present and discuss their proposals in tutored small working groups with other students. Other topics addressed in the course are how to construct a questionnaire, data collection, and data analysis. Several lectures are given about statistical data analysis, and each student gets his own random sample from a real dataset to analyze and to prepare tables for a manuscript. Concepts, such as bias and confounding, are explained in a few lectures, and students practice in recognizing problems in published studies in a tutorial on clinical epidemiology. Writing a report and how to get a paper published is a separate topic. Finally, research ethics are discussed, including examples of scientific misconduct, fraud, and plagiarism.

Electives

In the second and third year, all students choose four out of over forty available elective courses, which are either clinically, research, or organizationally oriented. This gives students the opportunity to choose topics they are particularly interested in or wish to orientate themselves in. Developing academic skills is a core element of all elective courses, including studying the literature, discussing interpretation with other students, writing a report and giving an oral presentation, and reflecting on the broader context. In total the students spend 12 weeks full time on these four courses. Alternatively, they can choose two courses from the list and spend six weeks on other topics of their own choice. This could be a course at another (non-medical) faculty at Leiden or another university, or making a start with a research project. This project serves as a mentored learning experience and could also be the preparation for their fourth year research project.

OPTIONAL TRACKS OF SCIENTIFIC TRAINING

Extra scientific training is offered to ambitious medical students. Currently three optional tracks are offered.

The *Excellence Track* is available for ten selected students. At the end of their first year these students are selected on the basis of motivation and high grades. In parallel to the medical curriculum they spend some 20% of their time on extra research under supervision at one of the departments of the LUMC. The selected students receive a small grant to visit scientific meetings abroad and can apply for a MD/PhD project for which two year of salary is provided by the LUMC.

An *Epidemiology Track* is available in which students can focus on clinical research and clinical epidemiology. In total, 12 weeks of extra courses on clinical epidemiology and biostatistics are offered. These courses are open to medical students as well as PhD students. Some courses have a classical one-week format with lectures and exercises, but recently also a master class on clinical epidemiology has been organized for which 60 students applied for only 30 available positions. This master class was organized during two weekends in a Youth Hostel. In a reading club, consisting of 14 meetings, the basic principles of the clinical epidemiology are discussed within a group of 20 medical students and two teachers. If students follow all these courses and do their scientific project in an epidemiological setting, they can apply for a formal registration as epidemiologist.

During the *Biomedical Sciences Track* students can follow a three-month premaster course in basic sciences. Upon successful completion, medical students can apply for a research master in Biomedical Sciences, which comprises one year of extra study, in addition to the clinical masters to become a physician.

THE RESEARCH PROJECT

Either just before or following their clerkships, each student does a research project for a period of 14-24 weeks full-time under supervision of a researcher. Projects are offered by most departments of the LUMC covering a wide range from basic research in a lab to translational, clinical, and epidemiological studies. Some 20-30% of the students do their research project either at another institute somewhere in The Netherlands or abroad. Before starting a student project, the research proposal must be approved by a scientific committee. Once approved, students work on their own research question which often fits in the context of a larger research project. They are involved in most phases of a research study, such as study design, data collection, and at least data analysis, interpretation, and report writing. Most students are also involved in research and other meetings organized in their department. The final report, usually in the format of a scientific paper, must be approved by the scientific committee to obtain a grade.

RESULTS OF THE LEIDEN MEDICAL TRAINING PROGRAM

As stated before, the scientific research training program is offered to prepare students for their research work and to encourage academic ambitions in students. This is accomplished by offering small pieces of directed education during the first 4 years of training. Integration with the medical content of the curriculum is essential.

One of the indicators for success is the number of students that take voluntary supplementary classes and voluntarily participate in research projects. In 2005 a survey was done among Leiden medical students in their fourth year, just before starting their clerkships. The web-based questionnaire was sent to 373 students of which 252 students responded (68%). 25% of the respondents reported being involved in additional research projects beside the compulsory medical school program. Most students started doing extra research in their third year. For them, academic ambition seems to be the strongest drive. Almost 75% of the students would have preferred more information on research possibilities and additional courses.

Furthermore, the survey showed that the students who are taking extra courses and are involved in research are not only the students with the highest grades. Therefore, it seems indeed useful to offer students extra education and research possibilities. Amongst other things, this survey was a stimulation to organize and promote more additional scientific education. This led to a substantial rise in applications for scientific courses.

Another indicator to the output of the program is the number of students that actually publish work in scientific papers. An evaluation in 2005 showed that almost 20% of all Leiden Medical School students were already authors on at least one full paper listed in PubMed on the day they completed their medical study. 22% of those students even were the first author of the paper. The average number of publications by these students was 1.5 articles per student. As a result of the usually substantial publication delay, the number of students who successfully prepared a scientific paper based on a student research project before their graduation is probably even higher.

FUTURE DIRECTIONS

Medical students in Leiden are enthusiastic about scientific research projects and do participate in many projects voluntary. Results show that many of them are involved and publish in scientific papers. In the upcoming years the Leiden scientific training program will be extended with some more courses to meet with the wishes of the students.

CONCLUSION

The LUMC has an extensive training program for scientific skills in place. The courses from the program are integrated in the preclinical curriculum. Based on the high number of students voluntary participating in scientific courses and scientific research projects, the LUMC scientific research training program for students is considered to be successful. Students are provided with

many opportunities to improve their academic skills, and many students actually take the opportunity. The number of medical students publishing in accepted peer reviewed journals is high.

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Structured Research Activity as a Vehicle for Fostering Reflective Practice among Medical Students

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ABSTRACT

Increasingly, medical educators are turning their attention and efforts toward enhancing the professionalism of medical students, starting with their first year of medical school [1]. Key to this endeavor is the development of educational structures and opportunities for ongoing reflection on practice. Scholars of how professionals in all fields think in practice, emphasize the continual cycles of reflection and action in which professionals engage as they hone their practice and improve the effectiveness with which they work with clients. Donald Shön, a leading figure in this field, speaks of reflection-on-action and reflection-in-action as hallmarks of professional habits of mind [2]. This article argues that structured research activities, woven into the medical school curriculum from the first year on, can foster the habits of mind associated with professionalism. Reflective practice can be seen to exist on a continuum with the more focused and rigorous systematic inquiry distinctive of research. Furthermore, this reflective practice is progressively entrenched and strengthened as students assume increasingly complex and autonomous roles in communities of inquiry, learning, and research [3, 4]. We present the research program at Case Western Reserve University School of Medicine (CWRU SOM) University Program as a model for how such student research activity situated in authentic research communities can be made an integral component of the medical school curriculum. The medical student research component of the curriculum has only this year been fully implemented, but we have already observed that the structured research activities of this curriculum, in conjunction with other aspects of the curriculum (including the use of a problem-based learning approach and a portfolio process), are effective in cultivating reflective habits of mind and engaging students in authentic ways in communities of inquiry and research, thereby strengthening medical student professionalism.

INTRODUCTION

REFLECTIVE PRACTICE AND THE PRACTICE OF RESEARCH IN MEDICINE

A hallmark of professionalism, as described by Donald Shön [2] in *The Reflective Practitioner*, is a habit of reflectiveness yoked to strategic action in one's field. As Shön puts it:

> The practitioner allows himself to experience surprise, puzzlement, or confusion in a situation which he finds uncertain or unique. He reflects on the phenomenon before him, and on the prior understandings which have been implicit in his behavior. He carries out an experiment which serves to generate both a new understanding of the phenomenon and a change in the situation. (Schön 1983: 68)

For the medical professional, the change in the situation desired is a positive change in the health status of the patient. Instead of calling it an "experiment," it would be more consistent with the ethics and mores of the medical profession to call the action a strategic action informed by best thinking in the field of medicine. Nonetheless, it has the same function as Shön's experiment: it both helps the professional taking the action improve his or her understanding of the phenomenon and changes the situation itself.

This is the key point: the medical professional integrates strategic action with an attitude of reflectiveness aimed at understanding, promoting, and evaluating a change in a patient's health status. In fact, if the action taken by the medical professional is to be truly strategic, it must, by definition of strategy, be informed by a systematic effort to understand the reasons for the changes in the patient's health status as a consequence of the action. Coming to this understanding of change requires the medical professional to engage in thinking about at least three essential aspects of the change: 1) to become clear about what is changeable in a patient's situation – that is, the parameters of health, well-being, and disease that have been or can be modified; 2) to explore the mechanisms and processes by which change occurs and the actions that can be taken to modify factors influencing the patient's situation; and 3) to be alert to the health outcomes and conditions that emerge as the change process unfolds. This is the fundamental thought process carried out in developing, implementing, and continually adjusting the diagnosis, treatment, and prognosis for a patient, but is construed more generally as a process of constructing an understanding of change in a patient's health status, which is at the heart of the strategic action informing, and emerging from, reflective habits of mind.

The medical professional's commitment to a focused and sustained effort targeted at understanding changes in the health status of a patient requires ongoing and systematic inquiry. This effort enmeshes the medical professional in repeated and often overlapping cycles of reflection and action that characterize professional thinking; a continual interplay is set up between reflection-in-action and reflection-on-action. Such systematic inquiry can exist in a continuum with more formal research activity: as questions or puzzlements arise in practice, these can be taken as fodder for focused research. They can be formulated clearly and explicitly – in measurable terms – as questions that drive research. It is, then, a natural next step for the medical professional to think of ways to gather data that will address his or her question as this inquiry unfolds. The professional analyzes the data, looking for clues to the nature of the change that he or she observes in the patient health status; the range of what can change and ways to characterize those changes; the factors that appear to bring about the changes; the measurable effects of action taken; and the conditions and results that emerge as the patient's health evolves. Thus, there has been an organic movement from more general reflection on clinical practice to more systematic studies of practice, which can take the form at one end of the continuum as formal research activity. All the action-oriented, inquiry-based activities on this continuum mutually reinforce and enrich each other in the context of reflective practice; put another way, action and reflection are seen as integral aspects of a more general reflective practice.

This view of research as existing on a continuum with reflective practice contrasts with an oft-heard belief that appears to set up an opposition between research and practice as separate enterprises. This misguided belief in the opposition of research and practice pervades the larger society as a bias against theoretical knowledge, caricaturing it as a diversion from "real-world" action. Shön's work is a withering critique of this attitude, identifying as it does the defining characteristic of a profession as the thoroughgoing and continuous integration of reflection and action. Under this view, if members of the medical profession – and aspirants to it, medical students – are to attain professionalism in their work, they must experience the synergy of participation in the creation of emerging knowledge from research and the never-ending honing of one's clinical practice

in the course of their medical careers. This may require a shift in their beliefs about the relationship between research and practice: they may need to move from believing that, in becoming doctors, they first amass a body of theoretical knowledge, demonstrate that they have mastered it in medical school, and then later apply this static, inert body of knowledge in the "real world" of their clinical practice (a belief which feeds back into the notion that research-based knowledge and effective clinical practice are discrete stages of professional growth and development) – to a belief that they are and should be active participants in the creation of knowledge in their field throughout their careers as they continually reflect on and improve their practice. It is this hope and philosophy that animates the medical student research program at Case Western Reserve University School of Medicine (CWRU SOM) University Program.[i]

Goals and Objectives of the Research Program in our Medical School Curriculum

It is in medical school that an orientation to reflection, systematic inquiry, and focused research can be fostered in students. It is the aim of the research component of the CWRU School of Medicine curriculum to cultivate such habits of mind, which will distinguish our graduates as medical professionals.

A number of behaviors make manifest this disposition toward structured reflectiveness. They arise from habits of mind that reveal a commitment to ongoing cycles of reflection and action; a critical but open stance toward emerging knowledge and research; a push to make inquiry increasingly systematic; a propensity to pose interesting questions that emerge from practice (so that issues encountered in patient care provide fodder for research); continually honing or even rethinking practice in light of new perspectives; relentlessly seeking root causes or underlying mechanisms for changes in patients' health status; and participating actively in the creation of new knowledge.

The desired goals for the medical student research program, aimed at promoting these habits of mind, include the following:

1. Valuing question asking

This is an attitudinal goal that affirms the importance of cultivating the reflective habits of mind associated with medical professionalism. The dimensions of this attitude include an understanding of the reflective habits of mind (a cognitive dimension); an appreciation of the value of these habits (an ethical dimension); and an orientation of one's behavior to these habits of mind (a dispositional dimension). The reflective habits of mind are worked into a framework of values that informs one's worldview.

2. Posing good questions

This goal is achieved when students can frame good questions for research, and appreciate the criteria for good questions. They work to make their questions clear and explicit; possess clinical and scientific relevance; lead to testable formulations of hypotheses; and offer new insight in a field. Students need to conduct an adequate literature review to stimulate good questions and to determine if the questions they generate are in fact worth pursuing. Students also keep their ears and minds open for good questions that may emerge in dialogue with peers and faculty members, in interacting with experts in the field, or in pondering puzzles or surprises in clinical encounters with patients.

3. Investigating questions systematically

Achieving the outcome of systematic investigation of questions will be reflected in student ability to formulate plans for their investigation. If they have framed good questions, then the proposal should reflect the formulation of clear, explicit, and testable hypotheses. The proposal should also reflect a working understanding of the range of research concepts, methods, techniques, instruments, and materials common in medical and biomedical research, and critical thinking about the methodologies that could best address the students' particular questions. At the end of our continuum of reflective practice, working on this goal means being able to investigate one's questions by conducting hypothesis-driven research, which becomes a key criterion in our assessment of fulfilling the requirements of the research component of the curriculum.

4. Constructing responses to questions with others in a community of inquiry

As students launch in earnest into an investigation of a question, they should continue to think critically about the strengths and limitations of the methodologies that they are employing (working in collaboration with their mentor/principal investigator and his or her team) and the inevitable trade-offs as one designs a research project. On an ongoing basis, students should reflect on and improve their research process, making refinements to (or even rethinking) hypotheses as needed, revisiting protocols, pondering emerging (and perhaps surprising) results, identifying

challenges or issues that arise, and engaging in ongoing problem-solving and reflection.

5. Communicating insights into questions to others in a broader community

In addition to contributing constructively to the community of learning, inquiry, and research of which one is a member as a researcher/investigator in a team, our students are also given opportunities to make contributions to the larger field or discipline which the research attempts to advance. Our students build skills in structuring their thinking in research as they learn to write proposals (both for their elective summer research experience and for their mandatory 4-month research block), compose structured abstracts for conferences, deliver presentations, and otherwise communicate their research in conference posters, research reports, grant applications, various articles, and other vehicles.

Key Components of Our Research Program

We help students work toward these goals by providing the following in the CWRU SOM curriculum: 1) the Foundations of Medicine and Health curriculum fulfills a number of research-related learning objectives, which assists students in preparing for their 4-month research block experience; 2) opportunities to conduct summer research after the medical school first year; 3) a four-month research block, where our students commit full-time to work with a mentor identifying research questions, framing methods, carrying out the research, conducting an analysis, and communicating their results in a write-up; 4) often (but not necessarily) building on their work in the four-month research block, students generate a medical thesis, writing up the results of their research in the format of a peer-reviewed journal in their field; and 5) students may take advantage of further opportunities to delve into research, including pursuing dual-degree programs and year-off fellowships. These five areas of the student four-year medical school experience are aimed at fostering professional habits of mind and inuring reflective practice. They represent a coherent developmental trajectory aimed at helping students achieve the above goals and provide a steady increase in challenge in terms of research knowledge and skills, independence in inquiry, and complexity of questions addressed, culminating with the medical thesis.

1. The Foundations of Medicine and Health curriculum

The Foundations of Medicine and Health curriculum, the foundational curriculum in basic and clinical sciences taken in the first year and a half of medical school, covers key concepts in biostatistics and epidemiology; evidence-based medicine; medical informatics; research methods and designs; population-based medicine; and ethical conduct in research, especially issues around informed consent. This curriculum focuses on giving students exposure to the design of clinical trials, the critical appraisal of research findings, the generation of hypotheses in research, the statistical analysis of data and interpretation of statistical measures, the conduct of systematic literature reviews, and an overview of the IRB process and approval.

A Research and Scholarship strand parallels all of the blocks of the Foundational curriculum. In each curricular block (e.g., Block 2 – The Human Blueprint, Block 4 – Homeostasis), researchers from across the campus are invited to speak to students on a bi-weekly basis about a research topic related to the theme of the block. These presentations represent a chance for students to learn more about the range of research opportunities available on campus, the key investigators and centers involved in the research area, and the research problems, methods, and techniques of importance to biomedical research. More subtly, these researchers often expose students to models of physician-scientists who have successfully contributed to research knowledge while remaining active in clinical practice. It is not uncommon for students who later conduct research in a particular field to have first heard about the research opportunity by attending one of these presentations

2. Summer research experience

CWRU School of Medicine incorporates active and meaningful participation in research as an integral part of its medical students' learning experience. At all steps in their medical school career, medical students are encouraged – and, in the case of the 4-month research block, required – to carry out research under the close mentorship of an experienced researcher in a field that is of interest to them. After their first year of medical school, medical students have a range of opportunities to engage in summer research during an 8- to 10-week period. The Office of Medical Student Research (OMSR) in the CWRU SOM has a full-time director that helps students identify summer research funding opportunities, including three NIH training grants and a private CWRU research fellowship (called the Crile

Summer Research Fellowship, available through the Crile Research Endowment at the school of medicine).

With the training grants and the Crile fellowship that OMSR administers, it is required that students take an active part in framing the research questions that they wish to pursue, formulating the methods that they plan to implement in order to investigate those questions, and defining the specific roles and responsibilities that they intend to fulfill in carrying out their research. Students must work with their prospective mentor to craft a proposal that they submit for review by the office. The students should negotiate with their prospective mentors the particular focus, questions, or hypotheses that will structure their study; of course, the work of the student fits in with the larger research agenda of the lab or clinic where they will work, but they define a niche for themselves within that larger context of inquiry. The proposal lays out: 1) an introduction stating what has been established in the field, open questions that remain, and the clinical relevance of the problem or question; 2) a clear statement of the research questions/hypotheses to be investigated; and 3) a section on the methods used for the investigation, including a full statement of the specific roles and responsibilities that students will carry out in the lab or clinic as they do their study.

In our review of their proposals, we place a heavy emphasis on ensuring that students have ownership in the research questions that they pursue based on an intellectual partnership with their mentors; that they develop intellectually substantive skills and knowledge in the conduct of research; and that they have meaningful roles in the research process. We want this proposal writing process to be a learning experience, so we offer coaching before they formally submit their proposals. Students involved in summer research are required to submit structured abstracts of their research at its conclusion. The summer research experiences help to build research knowledge and skills that will put students in good stead before they enter their more intensive and long-term 4-month research block.

3. Four-month research block

In the latter part of their 2nd year or their 3rd year, our medical students are required to make a full-time commitment of four months to conducting research as part of a mentored learning experience, carrying out an in-depth investigation of a problem or issue that is empirical and hypothesis-driven. There are four different times at which students can schedule their research blocks to fulfill this requirement. Students are assisted by OMSR, the deans of their academic societies, the Vice Dean of Research, and other faculty members in selecting appropriate mentors, matching their research interests to appropriate investigators in laboratories or clinics. The sites for their research may be at CWRU School of Medicine or any of its affiliated institutions, which includes a prestigious private clinic with an active and wide-ranging research program, an academic medical center, a large urban hospital with a significant needy clientele, and a Veteran Affairs medical center. With all of these sites available for research, there is no shortage of appropriate research opportunities for students that provide a good match with student interest and background. Students may also opt to work in a lab or clinic outside of CWRU School of Medicine, given that they fulfill the requirements of having their research proposal approved and of naming an appropriate co-advisor affiliated with CWRU SOM.

As with summer research, we ensure that the student involvement in the research is high-level and meaningful, but their role now requires more autonomy in shaping the inquiry, responsibility in generating results, and accountability in creating intellectual products (especially a research summary that will usually become the basis for their medical thesis). Generally speaking, students affiliate themselves with principal investigators whose research activity is ongoing or have a project that is soon to be launched, complete with institutional review board (IRB) approval. Students work with their prospective mentors to carve out a set of questions or hypotheses in which they have ownership, but which also meshes with the larger research agenda of the laboratory or clinic. Working under the guidance of their research advisor, students craft a proposal that lays out the importance of the question or issue that they intend to investigate; clearly and explicitly defines the hypotheses or questions to be tested; and specifies the methods that they will employ to test these hypotheses. They must also conduct a preliminary literature review to gain an understanding of the importance of the topic, what has already been established in the field, and how their own research question relates to other open questions in the domain. They conclude the proposal by describing in detail the specific roles and responsibilities that they will fulfill in carrying out the research. Since they have moved along a developmental trajectory in building their research knowledge and skills, we expect a more detailed research proposal than is expected for students

conducting summer research after their first year of medical school.

The research performed for the 4-month research block must meet certain criteria. Foremost, the research must be hypothesis-driven: there must be a hypothesis (or set of hypotheses) that is empirically testable, and all other aspects of the research must be aimed at investigating the hypothesis. Students must be working with original, largely unprocessed data. While the research fits in with the aims of the laboratory or clinic in which they are conducting their research, they must find a niche for themselves within this work and take ownership over questions that will drive their own investigation. This requires that students negotiate their research focus with their prospective mentors before starting their research block. Students must determine that the institutional review board (IRB) approval will be in place in a timely manner, so that their work during the block is not impeded. Occasionally, we have students who wish to pursue an original research idea. If this is the case, we fully support the student, but make him or her aware that they will need to start planning for their de novo research project well before the start of the 4-month block, that they will need to find a mentor who sponsors them in the project and is willing to provide intensive mentorship and guidance as they formulate and implement the project, and that they will need to work with this mentor to put in place IRB approval before the start of the block. Such highly individualized research will likely require time beyond the 4-month block to finalize the data gathering and analysis.

At the conclusion of the block, students are required to submit a summary of their research. This summary can be seen as a working draft of the medical thesis that they are required to submit in order to graduate in the 4th year of medical school. Some students opt to continue the research endeavor that they began in their research block in order to gather more data, complete the analysis of data collected, or work with their mentors to put their research into a publishable form. One-month research electives are available for them to complete their research, if necessary.

4. Medical thesis

Typically, the work that students carry out in their 4-month research block becomes the basis of their medical thesis. The format and content of the medical thesis should emulate an article as it would appear in a peer-reviewed journal in their field. Thus, the term "thesis" may be a misnomer, since it is unlike a thesis one would write for graduate school, but the term has become entrenched at the medical school ("research report" might be a better term).

At this point in the student medical school career, there should be no surprise that the thesis must be based on empirical research that is hypothesis-driven. The intent of this learning experience is to simulate a peer-review process for students, granting them a sense of what it is like to publish research in the field of medicine. We have assembled a medical thesis review committee, composed of researchers with a range of expertise to match the variety of medical thesis topics that students submit, who apply criteria that are the usual criteria one would find applied in selecting articles for any peer-reviewed journal. In general, these criteria track the key sections of a prototypical journal article: 1) is the overall research hypothesis-driven?; 2) are clear and testable hypotheses presented?; 3) are replicable methods described that are well-targeted to testing the hypotheses?; 4) do the results presented serve to confirm or reject the hypotheses?; 5) is there a forthright discussion of the strengths and limitations of the methodology used?; and 6) are the key findings supported by the evidence in the report? (The appendix presents in detail the criteria that our reviewers use.)

There are students who do, in fact, work with their mentors to publish an article in a journal. Naturally, this is encouraged; we see such student research productivity as a key indicator of the success of our medical student research program. If students publish their research as a co-author, they must establish that they had a significant role in the authorship of the manuscript and their mentor needs to attest to this. It is also possible for students to submit a thesis that is a product of research effort conducted while in medical school but not connected to their research block project. The "peer reviews" provided by faculty are shared with students, and they are given a chance to revise their thesis to meet the requirements of the thesis.

5. Further opportunities for research: Year-off opportunities, dual degree programs, and options for research electives

We have students who become so engrossed in research that they decide to pursue a year-off to delve deeply into a problem or issue of great interest to them. Our program allows for students to take a year-off, and OMSR works with students who are interested in participating in year-off research identify and compete for funding opportunities, such as fellowships from the Howard Hughes Medical

Institute, Sarnoff Foundation, the Doris Duke Foundation, or Fogarty International.

Finally, students may pursue a dual degree option to expand their opportunities to pursue an area of research interest in greater depth and deepen their skills and understanding in a specialization. These dual degree programs include an MD/MA in Bioethics, an MD/MPH, an MD/MS in Anatomy, an MD/MS in Biomedical Engineering, and an MD/MS in Biomedical Investigation.

Lessons Learned and Future Directions

The research component of our curriculum pitches a student forward on a developmental trajectory, where the student moves from peripheral participation in a community of researchers to increasingly active, complex, and autonomous roles in such communities as they take on more challenging research activity and further develop their research skills and knowledge [3, 4]. We have faced a number of challenges in helping students move along this trajectory; examining these challenges will inform our improvement efforts as we act on enhancing student preparation for productive and meaningful engagement in their research block.

First, the Foundations of Medicine and Health curriculum needs to be strengthened to address difficulties that students often encounter as they formulate their research proposals for their summer or research block experience. More attention will be given to developing student skills in generating and formulating hypotheses; in bolstering their understanding of appropriate statistical measures and how to interpret them; and in increasing their ability to critique – and even rethink – the methodologies they employ in carrying out their research. This is especially vital for those students who have not conducted research previous to matriculating at our medical school.

It is hoped that strengthening these aspects of our curriculum will reduce the occurrence of two common problems that we see in reviewing research proposals: 1) failure to clearly and explicitly articulate hypotheses; and 2) omission of a detailed description of the student specific roles and responsibilities in carrying out the research (some students only provide highly generic language about how research is performed in the clinic or laboratory).

As the students move into their research block, the mentorship and support that their principal investigator provides to them becomes critical. We are looking at ways to bolster the assistance and support we provide to mentors, and to improve communication of the expectations that hold for their work with students, articulating clear roles and responsibilities for them. Student research block experience is viewed as a mentored learning experience, and the meaningfulness, engagement, and intellectual substance of the experience depends critically on the mentor skills in modeling and coaching research thinking and activity, as needed for the student. Mentors need to work with students to heighten their intellectual ownership of the work being performed, prompting them to pose worthwhile questions, think critically about methodologies, improve protocols, analyze and represent data, interpret results, consider alternative explanations of data, and learn how to communicate their research to others. Effective mentors look for ways to heighten student intellectual engagement and to help students take advantage of multiple opportunities to present and publish their results (e.g., attending conferences, co-writing articles, submitting abstracts, writing grant proposals, documenting IRB processes).

In addition to the support of mentors, we are planning to put in place other supports that will better prepare students for the research block. We wish to help students access resources in statistical and research design consultation as they plan for, implement, and evaluate the results of the research performed during their block. We want to provide opportunities for students to "workshop" their proposals before entering the block, speaking with other students and faculty experienced in research methodology to think more deeply about their hypotheses and the research designs to test them. During the block, we are planning workshops targeted at high-priority skills and processes in research activity (IRB process/approval, writing of proposals or structured abstracts, effective poster design, etc.) In this way, we want to create an even stronger support system to better ensure that all students in our medical school curriculum have a meaningful, high-quality mentored learning experience for their research block. Such a support system will help students integrate the research block experience more effectively and coherently with the rest of the medical school curriculum. This, in turn, will promote the shift in attitude we wish to see in students: an attitude where clinical practice is not seen as divorced from research, but part of the continuum of reflective practice that should pervade inquiry, whether it is situated in a clinical setting or a research setting.

The culmination of this developmental trajectory in research skills and knowledge comes with the writing and submission of the medical thesis. Here, we wish to help students work on clearly stating their focus and research question, engaging in an adequate critical discussion of the strengths and weaknesses of the study methods, and offering conclusions that are properly qualified in their scope given the evidence gathered and analyzed. We hope the strengthening of the research component of the curriculum, careful mentorship, and the provision of wider support systems will better ensure that all students are producing high-quality theses meeting or exceeding the criteria listed in the appendix.

Our medical thesis reviewers need to be clear that their function, at the point when the thesis is submitted (in early January of the student's fourth year of medical school) is to provide a "peer review" of the thesis, rather than mentorship. Our medical thesis reviewers, especially those who are not M.D.s but are Ph.D.s, need to learn to recognize that this thesis differs from those that would be submitted by students working on a master or doctoral degree in basic science programs. They need to adjust their expectations in evaluating the research reports of our medical students, submitted as medical theses.

Finally, for those students submitting articles that they co-authored with their PIs, we need to make clear that they had substantive roles in writing the manuscript, establishing that it is legitimate for them to claim a significant portion of the work as their own.

CONCLUSION

The structured research activities in which students are engaged throughout their medical school career at CWRU SOM are aimed at cultivating the reflective habits of mind associated with professionalism in medicine. Increasingly, students over the course of their medical school career at CWRU SOM are engaged in more complex and self-directed research efforts that draw them from the periphery of working in communities of research and inquiry into the core of such communities. It is in this activity that students see modeled, and put into action, reflective practice, practice that integrates reflection and action as aspects of the same professional orientation. This reflective practice is reinforced and extended by other aspects of the medical student curriculum, including the dialogue and reasoning centered on the cases that students work through in their problem-based learning sessions (a key part of our curriculum) and the portfolio processes in which they engage.

It has been argued in this manuscript that the habits of mind so intimately bound up with medical professionalism are precisely those habits of mind fostered in the activity of research. It is the aim of CWRU SOM research component in the curriculum to provide the opportunities, structure, and environment for its medical students to engage in meaningful research, with the ultimate hope of developing students into lifelong learners inured to these habits of minds. In this way, our medical students will emerge from their medical school experience with a clear sense of what it means to be a physician-scholar, a professional committed to ongoing cycles of reflection and action in their careers and to continual, systematic inquiry, moving toward more structured research activity in their practice as appropriate and enriching, but always engaged in reflective practice.

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[i] The CWRU School of Medicine has two tracks: the University Program, whose research program this article will describe, and the Lerner College Program, housed at the Cleveland Clinic Foundation.

APPENDIX: MEDICAL THESIS ASSESSMENT FORM

Assessment	Yes	No	N/A
Overall			
The medical thesis follows the format of articles that appear in peer-reviewed journals in the chosen field.			
The research supporting the thesis is hypothesis-driven.			
Background / Introduction			
The medical thesis reflects an understanding of the important questions, open issues, and most salient established results in the chosen area of research, grounded in an adequate review of the literature.			
The thesis makes a convincing argument that the research questions pursued may contribute to understanding health and disease.			
The thesis makes a convincing argument that the research questions pursued are worthwhile in addressing clinically relevant needs.			
Hypothesis / Hypotheses			
There is a specific and clearly formulated hypothesis or set of hypotheses stated in the theses.			
The hypothesis/hypotheses is/are testable.			
Methods			
The thesis research is based on methods or techniques in the chosen field of study which are considered sound.			
The methods or techniques used in the research are well-targeted to testing the hypothesis/hypotheses.			
The thesis describes the protocol used in the research with adequate detail, such that it would be reasonable that another researcher in the field could replicate the study with fidelity.			
Results			
The thesis presents new data that serve to support or reject the stated hypothesis/hypotheses.			
The data presented were subjected to rigorous and systematic analysis that allowed for the confirmation or rejection of the stated hypothesis/hypotheses.			
Discussion			
The Discussion section situates the results of the research in the larger context of inquiry in the relevant research community.			

The Discussion section forthrightly presents the strengths and limitations of the current study.			
The Discussion section suggests promising directions for future research and compelling questions that remain.			
Conclusion			
The thesis presents a Conclusion section that succinctly summarizes the key findings of the study. [This may be included in the Discussion section, but assess separately nonetheless.]			
The claims made in the key findings are justified by the evidence presented in the thesis.			

LIMSC, a Student's Key to the Future

Since the scientific community is rapidly internationalizing, students in the life sciences or medicine, need to be increasingly aware of the international character of their future careers. For this purpose the Student Board of the Leiden University Medical Center (LUMC) organizes biannually the Leiden International Medical Student Conference (LIMSC). Students from all over the world convene for this three-day conference in Leiden. At this conference students can broaden their professional outlook by meeting their fellow student researchers in an informal setting, visit guest lectures by prominent international researchers and take part in interactive workshops. During the sixth edition of LIMSC in March 2009, we were proud to welcome amongst others Professor Sir R. Maini (Professor of Rheumatology, Imperial College London, United Kingdom) and Prof.dr. C.J.H. van de Velde (Professor of Surgical Oncology, Leiden University Medical Center, The Netherlands).

Students can participate in LIMSC either as a presenting or as a visiting participant. Students from all over the world were invited to submit an abstract of their research. In the last edition, over 600 students submitted abstracts about a wide variety of (bio)medical topics, ranging from internal medicine and public health to molecular cell biology and ethics. The 200 best abstracts were selected by a blinded committee of Professors of the LUMC, based on the quality of research, the originality of the research, and the quality of the abstract. The best 120 students were invited to give an oral presentation and the other 80 students gave a poster presentation.

LIMSC 2009 has been a big success! We are proud the give you for the first time the opportunity to take a look in the selected 200 abstracts of the presenting participants of LIMSC 2009. For more details about LIMSC, please visit www.limsc.nl.

Organizing Committee LIMSC 2009

CARDIOLOGY

Acute Myocardial Infarction (AMI) Versus Posterior Myocardial Infarction

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Introduction: It is known that patients with anterior MI usually have worse outcome and prognosis compared with those with inferior (posterior) MI.

To evaluate the outcome and prognostic significance of patients with AMI depending on it's location (anterior vs inferior), patient's sex , LVEF and the relation between them.

Material and Methods: 43 patients with diagnosis of AMI were examined and investigated in a period of 3 weeks.
They were all examined with a routine clinical tests and specific methods of investigations were performed to estimate infarction's location, its complications and the level of LVEF.

Patients were grouped according to location of the infarction(anterior 67% (n=29); inferior 33% (n=14)), the patient's sex (men 61% (n=26); woman 39% (n=17)), the level of LVEF(61% of patients had EF<40%and 39% had EF>40%).

Results: It was found that most patients with anterior MI had a substantially worse outcomes and prognosis(60% were presented with complications and low LVEF 38%) compared with those with inferior MI(16% had complications with LVEF 43.7%).Complications in patients with AMI includes: conduction defects18%; aneurysm 12%;arrhythmia10%;and acute pulmonary edema 4%. Also it was revealed that 80% of men with AMI were having complications compared with woman only 40%.And mostly patients with MI complications(65%) demonstrated a low level of LVEF(33%) which correlates with left ventricular systolic dysfunction.

Conclusion:

1. Patients with anterior MI usually have worse outcome and prognosis compared with those with inferior (posterior) MI.
2. Men with AMI develop complications more often than in woman (ratio 2:1).
3. Appearance of complications in patients with AMI is connected with systolic dysfunction of the left ventricle.

CARDIOLOGY

The Association Between Serum Total Cholesterol Level and Deaths Caused by Coronary Heart Disease

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Introduction: Coronary heart disease is the first cause of death in the whole world. The serum total cholesterol level predicts the risk of coronary heart disease in middle-aged men, but results are inconsistent in studies on serum cholesterol and mortality among the elderly. This study was done to determine the relation between the serum total cholesterol level and the mortality caused by coronary heart disease.

Material and Methods: A surveillance study was done in Tehran to identify cases of the changes in serum cholesterol level and mortality during a 3 year period (2004-2008) among 646 50-75 year old men and women who were asymptomatic of coronary heart disease in 2004. These patients referred to Imam Khomeini hospital for symptoms not related to coronary heart disease. Changes in serum cholesterol level were computed based on measurements made in 2004, 2006 and 2008 and were requested to supply us with their past tests if any. Patients participating in this study were followed by phone to look for any deaths occurring in the time of the study.

Results: 168 patients (26%) died during a 4 year period. 93 deaths (55%) were caused by coronary heart disease, 38 deaths (22%) were caused by cancer and the remaining deaths were related to other causes including traffic accidents, falls, suicide and burns. Among 397 patients aged 50-60, 67 died of coronary heart disease. Among these patients, 63 had cholesterol levels higher than 250 and 27 higher than 400. However, the relation between serum cholesterol levels and the mortality caused by coronary heart disease in elderly patients was not this significant.

Conclusion: There is a significant association between serum total cholesterol level and deaths caused by coronary heart disease in 50-60 year old patients. However, among older patients other factors such as aging and increased incidence of chronic diseases seem to be responsible for a significant proportion of deaths. This study helps to explain why some previous studies have not found an association between serum cholesterol level and deaths caused by coronary heart disease among elderly. We believe that government should take strong actions to reduce the risk factors of coronary heart disease by changing the modern lifestyle, and for these actions to work, they ought to be started from early stages of life.

CARDIOLOGY

Genetic Variation in Fibrinogen; its Relation to Fibrinogen Levels and the Risk of Myocardial Infarction and Ischemic Stroke

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Introduction: Confounding by common causes and reverse causation have been proposed as explanations of the association of high fibrinogen levels and cardiovascular disease. Genetic variants can alter fibrinogen characteristics and are not subject to these problems.
To determine the fibrinogen plasma levels for genotypic variants in fibrinogen-alpha (FGA6534, Thr312Ala) and fibrinogen-beta (FGB1437, G-455A) and whether these variants are associated with arterial thrombosis.

Material and Methods: Fibrinogen genotypes were determined in a population based case-control study including women aged 18-50 years; 218 cases with myocardial infarction, 192 cases with ischemic stroke and 769 healthy controls. Fibrinogen levels were determined in the control population.

Results: Carriers of the FGB1437 carriers of the rare allele (-455A) had higher fibrinogen levels than carriers of the common allele. For FGA6524 carriers of the common allele (Thr312) fibrinogen levels were also increased, albeit to a minor extent. When comparing the homozygote carriers of the high fibrinogen allele to the homozygote carriers of the low fibrinogen allele the odds ratio and corresponding 95% confidence level of myocardial infarction did not increase (1.22; 0.72 to 2.06 for FGA6534/Thr312 and 0.82; 0.49 to 1.39 for FGB1437/-455A). The risk of ischemic stroke was increased for both the FGA6534/Thr312 (2.32; 1.15 to 4.69) and FGB1437/-455A variant (1.76; 0.7 to 4.03).

Conclusion: With the genetic variations as markers of increased plasma fibrinogen levels (especially FGB1437/-455A) to rule out confounding and reverse causation these results suggest plasma fibrinogen levels are more important as risk factors for ischemic stroke than for myocardial infarction.

CARDIOVASCULAR

Quality of Life in Patients of Different Age Groups Six Months after Coronary Artery by-pass Surgery

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Introduction: Examination of the preoperative and postoperative quality of life relation to the patients' (pts) age - group and determination weather the patient's age could be predictor of the QOL changes after CABG.

Material and Methods: From February to May 2002, we administered the Nottingham Health Profile Questionnaire part I in a consecutive series of patients (243 pts, 195 men) who underwent elective CABG. It contains 38 subjective statements divided into six sections: physical mobility (PM), social isolation (SI), emotional reaction (ER), energy (En), pain and sleep. The questionnaire was distributed before and six months after CABG, to all patients.

Results: Four age groups were studied: < 50 years (36 pts, 14.82%), 50 to 59 years (87 pts, 35.80%), 60 to 69 years (98 pts, 40.33%), and 70 years or older (22 pts, 9.05%). In two higher age groups we fond significantly worse scores of preoperative QOL in sections of PM (p=0.023) and En (p=0.005) comparing with two younger groups. Six months after operation the QOL was improved in 53.75% of cases. The improvement in QOL was related to the patients' higher age group in section of PM (r=0.177, p=0.008), SI (r=0.169, p=0.011) and En (r=0.209, p=0.002). We found that the younger age was the independent predictor of the QOL improvement in the section of pain, six months after CABG (p=0.013, odds ratio = 0.629, 95% confidence interval 0.436 to 0.907).

Conclusion: Older patients had worse preoperative QOL. More significant improvement was registered in patients who had worse QOL preoperatively (older pts). Younger age is the independent predictor of QOL improvement in the section of pain after CABG.

CARDIOVASCULAR

Advanced Glycation Endproducts and Their Role in the Field of Vascular Surgery

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Introduction: The formation of advanced glycation endproducts (AGEs) is the result of the non enzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids. They represent the cumulative burden of hyperglycaemia, hyperlipidaemia, and oxidative and carbonyl stress. Previous studies have shown direct relations between level of AGEs as detected by skin autofluorescence and progression of vascular stenosis and plaque vulnerability, and they predict future cardiovascular morbidity and mortality. This study intends to examine the exact role of AGEs in the field of vascular surgery. First of all, is the level of AGEs increased in patients suffering from severe atherosclerotic disease? Furthermore, the skin AGE level differences between aneurismal and atherosclerotic occlusive disease will be examined, as well as the differences between preoperative and postoperative patients.

Material and Methods: Between September 2007 and February 2008 non-invasive AGEs measurements were proposed to all patients attending the out-patient clinics of the Division of Vascular Surgery of our hospital. The tissue accumulation of AGEs was measured with an AutoFluorescence Reader (Diagnoptics BV, Groningen, The Netherlands), designed to noninvasively and rapidly measure skin auto fluorescence and so AGE accumulation. Patients with diabetes or varicose veins were excluded from this study.

Results: The present study group consisted of 359 patients, 275 male (77%) and 84 female (23%) (ratio 3:1). The average age was 67 years, ranging from 23 to 89 years. Regarding the first aim of this study, our study group showed a higher level of skin AGEs than the normal distribution (2.74 arbitrary units (AI) vs. 2.38, respectively). With a p value smaller than 0.001, this is a significant difference.

No difference (p=0.44) was found between aneurismal (n=121, 2.71) and atherosclerotic occlusive (n=214, 2.74) disease.
With regard to the third aim of the study, approximately half of the study group (n=193, 54%) underwent vascular surgery before AGEs measurement. Although a slight difference between preoperative (2.71) and postoperative (2.77) values was found, this difference was not significant (p=0.40).

Conclusion: It can be concluded that AGE accumulation is increased in patients suffering from severe atherosclerotic disease. This implicates a strongly raised cardiovascular risk and increased mortality. These AGE levels however, do not seem to be affected by the nature of vascular disease, nor by a surgical intervention.
Further evaluation will be needed to define the exact role of AGEs in the field of vascular surgery.

CARDIOVASCULAR

Preoperative Statin Therapy in Surgical Ablation of Atrial Fibrillation in Patients Undergoing Concomitant Cardiac Surgery

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Introduction: Statin therapy reduces the incidence of atrial fibrillation (AF) after cardiac surgery through anti-inflammatory effects. We hypothesized that statin use prior to surgical ablation for AF during concomitant cardiac procedures can improve early ablation outcomes.

Material and Methods: One-hundred patients (69±10 years;69% men) with paroxysmal (34%) or permanent (66%) AF undergoing cardiac surgery (CABG 55%, valve surgery 65% and aortic surgery 4%) and surgical ablation were retrospectively analyzed. Applied energy sources for AF ablation were radiofrequency (84%) and ultrasound (16%). Fifty patients (50%) were on preoperative statins (statin group) before surgery. Early conversion rates to SR (OR, ICU, hospital discharge, 3 months), freedom from anti-arrhythmic therapy and pro-inflammatory C-reactive protein (CRP) levels were recorded.

Results: Intraoperative conversion rate to SR was 92% and 94% in statin and control patients. SR was preserved in 82% vs. 80% of patients at ICU, 52% vs 56% at discharge and 55% vs 62% at 3 months in statin pretreated and control patients (p=n.s). Similarly, there was no difference in electrical cardioversion rates (8% control vs 16% statins) and freedom from antiarrhythmic therapy (56% control vs 42% statins) between treatment groups. Preoperative statin use was not linked to reduced CRP levels after surgical ablation (maximal CRP 165±68 (control) vs. 177±67 mg/dl (statin); p=n.s.).

Conclusion: Our preliminary data suggests that preoperative statin therapy is not associated with improved early ablation outcome in patients undergoing cardiac surgery with surgical ablation for AF. Data from RCT with extended follow-up periods are needed to allow definite conclusions.

CARDIOVASCULAR

Ultrasound Anatomy of Venous System of Posterior Compartment of the Thigh

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Introduction: Disturbances of venous outflow from the posterior compartment of the thigh can result in formation of varicose veins and teleangiectasias on the posterior aspect of the thigh. In case of increased venous pressure in veins of sciatic nerve, patients often complain of the symptoms of ischialgia due to sciatic nerve ischemia. Presence of sciatic perforating veins and cranial extension of short saphenous vein (CESSV) can promote the recurrence of varicose veins. Diagnostic imaging of these vessels bases nowadays on ultrasound visualization accompanied by Power Doppler and Color Doppler. The study aimed to describe the most common patterns of venous outflow from the posterior muscular compartment of the thigh with a special attention to CESSV.

Material and Methods: Research was carried out on ultrasonographic examinations of 42 lower extremities of 21 healthy volunteers aged 20-34 years (12 males, 9 females). Ultrasonographic examinations were done using GE Voluson 730 Expert scanner equipped with 7,5 MHz linear transducer in B-mode, including PW Doppler, Power Doppler and Color Doppler studies. In case of problems in identification of a vessel compliance test and Power Doppler were used.
Results: Ultrasound studies showed the presence of CESSV in 37 limbs (20 right, 17 left). In 11 cases (7 right, 4 left) it continued toward great saphenous vein as intersaphenous vein of Giacomini. Subfascial course of CESSV with its drainage into deep veins of the thigh was observed in 17 extremities (11 right, 6 left). It was impossible to determine exactly in which of deep veins CESSV terminated. In 9 cases (2 right, 7 left) the CESSV anastomosed epifascially with superficial veins and disappeared on posterior surface of the thigh.

Conclusion: (1) CEESV was present in majority of examined extremities. (2) CESSV can be visualized using ultrasound examination. Power Doppler and Color Doppler are useful to ease visualization and assess direction of blood flow. Course of CESSV is easy to visualize especially in cases where Giacomini's vein is present. (3) Termination of CESSV draining to deep venous system is usually difficult to visualize.

CARDIOVASCULAR

The Relationship Between a Novel Tissue Doppler Index and NTproBNP Levels in Patients with Left Ventricular Dysfunction

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Introduction: N-terminal pro-brain natriuretic peptide (NTproBNP) is recognized as a reliable marker of systolic and diastolic left ventricular (LV) function. The ratio between early diastolic transmitral velocity and early mitral annular diastolic velocity (E/Ea) reflects LV filling pressure in a variety of cardiac diseases. However this relationship was not validated in some categories of patients. We belive that combining the index of diastolic function (E/Ea) and a parameter that explores LV systolic performance (Sa, peak systolic velocity of mitral annulus) provides a close prediction of NTproBNP.

The aim of this study is to assess the relationship between a new parameter, E/(Ea×Sa), and NTproBNP level in patients with LV dysfunction.

Material and Methods: We screened 145 consecutive patients with LV dysfunction in sinusal rhythm referred for LV cathetherism. Patients with inadequate echocardiographic image, paced rhythm, mitral stenosis, significant primary or organic mitral regurgitation, mitral prosthesis, severe mitral annular calcification, pericardial disease, acute coronary syndrome, coronary artery by-pass within 72 hours or renal failure were excluded. The remaining 113 patients (78 with heart failure with reduced ejection fraction –HFREF, 12 with HF with normal EF, 23 with isolated diastolic dysfunction) formed our study group. Echocardiography was performed simultaneously with NTproBNP measurement. E/Ea and E/(Ea×Sa) were calculated; the average of the velocities of septal and lateral mitral annulus was used.

Results: Simple regression analysis demonstrated a significant linear correlation between E/(Ea×Sa) and NTproBNP (r=0,70, p<0.0001), superior to E/Ea correlation (r=0,57, p<0.0001). Significant but weaker correlations were found between NTproBNP and pulmonary artery systolic pressure (r=0,52, p<0,0001), Sa (r= -0,44, p<0,0001), LVEF (r=-0,33, p=0,001), E wave (r=0,32, p=0,001), mitral E deceleration time (r=0,32, p=0,001) and Ea (r=-0,26, p=0,007). We couldn't demonstrate significant relationships between NTproBNP and left atrial (LA) diameter, LA surface or LA volume. The optimal E/(Ea×Sa) cut-off for prediction of NTproBNP levels >1200 pg/ml was 1,6 (sensitivity=83%, specificity=75%). Among analyzed parameters, E/(Ea×Sa) was best correlated with NTproBNP levels in patients with HF with normal EF (r=0,77, p<0,0001), in those with HFREF (r=0,65, p<0,0001) and in those with isolated diastolic dysfunction (r=0,60, p<0,0001).

Conclusion: E/(Ea×Sa) had a good correlation with plasma NTproBNP level and can be a simple, reproductible and accurate echocardiographic index in patients with LV dysfunction in sinusal rhythm.

CARDIOVASCULAR

InflammatoryResponse to Myocardial Ischemia Reperfusion Injury in Patients with and Without Pre-existing Heart Failure

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Introduction: Myocardial Ischemia Reperfusion Injury (MIRI) is the paradoxical exacerbation of myocardial damage upon restoration of blood flow to previously ischemic myocardial tissue. MIRI is held responsible for Systemic Inflammatory Response Syndrome (SIRS) after cardiac surgery. For unknown reasons is SIRS more common in patients with pre-existing heart failure. We hypothesize that this discrepancy is caused by a different myocardial inflammatory response to MIRI. In a pilot study we examined the local inflammatory response in patients with and without pre-existing heart failure, and we determined the feasibility of the study and the optimal times for blood sampling.

Material and Methods: Arteriovenous concentration differences were used to specifically measure myocardial inflammatory responses. Paired blood samples, of five patients with and five without pre-existing heart failure undergoing mitral valve surgery, were obtained at fixed time-points until twenty-four hours after reperfusion by using a coronary sinus (CS) catheter. For a comprehensive analysis we measured a panel of cytokines using the Bio-Plex human cytokine 27-plex panel to investigate inflammatory pathways. Endothelial activation was established by ICAM-1 and vWF.

Results: Feasibility of sampling out of the CS was confirmed by measurement of oxygen saturation, which was in CS blood almost 10% lower than in central venous blood. Significant release of IL-1β (p = 0,036), IL-6 (p = 0,001) and IL-9 (p = 0,005) twenty-four hours after reperfusion was shown. Our study did not reveal significant differences for other cytokines, neither arteriovenous nor between patients with and without pre-existing heart failure. Endothelial activation was not established by measuring ICAM-1 and vWF.

Conclusion: Feasibility of sampling from the CS and optimal times for blood sampling were confirmed with this pilot study. Arteriovenous differences for IL-1β, IL-6 and IL-9 were found, indicating a cardiac origin of these cytokines. Indications for plasma concentration differences of cytokines between patients with and without pre-existing heart failure were found, but the differences were not significant. The hypothesis that a different response to MIRI underlies the more frequent development of SIRS in patients with pre-existing heart failure needs further investigation.

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CARDIOVASCULAR

Warming Due to Hydration of DMSO is the Cause of Hypotension Following Intra Peritoneal Injection in Rat

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Introduction: Dimethyl sulfoxide (DMSO) is one of the most common solvents of organic substances and has multidisciplinary utilization due to its therapeutic effects and several laboratory properties. Some cardiovascular side effects of DMSO have been reported. We accidentally found that it has a major hypotensive effect. In this study we investigated the mechanism of hypotesion following intra peritoneal injection of DMSO.

Materials & Methods: In urethane anesthetized rats, direct measurement of arterial blood pressure and intra peritoneum temperature were performed. We investigated the effects of DMSO on blood pressure by intra peritoneal (IP) injection of 1gr/kg pure DMSO (100%) or saline diluted DMSO (80%, 60% and 50%) in a way that in all cases equal amount of DMSO was injected. In other group, 3ml of 42ċ normal saline was injected for evaluation of peritoneum warming on blood pressure. Following all injections, arterial blood pressure and intra peritoneum temperature were measured. We also investigated the effects of pre-treatment with histamine receptor antagonists (Chlorpheniramine 4mg/kg), nitric oxide synthase inhibitor (L-NAME, 40 mg/kg) or vagotomy on hypotensive effects of DMSO and warm saline.

Results: This study shows that the IP injection of 100%, 80% and 60% of DMSO lead to ±5, ±6 and ±4 reduction in mean arterial pressure respectively, accompanied by increasing of the intra peritoneum temperature at the range of 3±0.2 ċ,1.5±0.1 ċ and 1.2±0.3ċ respectively. The 50% DMSO didn't change blood pressure or peritoneum temperature. Pre-treatment with L-NAME and vagotomy partially attenuated effects of non hydrated DMSO and warm saline on blood pressure but cholorpheniramine inhibited this effect completely.

Conclusion: According to our results, DMSO hydration in peritoneum increases temperature that results in mesenteric vascular dilation and hypotension by reduction of peripheral resistance against blood flow. Mesenteric thermoregulatory mechanisms are mediated by local release of histamine and nitric oxide and/or neural parasympathetic reflexes. IP injection of warm saline causes similar effects to DMSO, whereas completely hydrated DMSO has no effect. This study for the first time reveals the biological effects of DMSO which is due to its thermogenic properties following hydration. Thermogenic hydration of DMSO is the probable cause of some side effects, such as bradycardia, heart block, cardiac arrest and hemolysis. Regarding to frequent

clinical use of DMSO, further investigation is needed to clarify concern about this phenomenon.

CARDIOVASCULAR

The Effect of Psychosocial Factors on the Recovery of Patients after Cardia Surgery

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Introduction: Anxiety has been shown to predict long-term mortality after myocardial infarction and cardiac surgery. We
have hypothesized that psychosocial factors contribute to in-hospital mortality and morbidity.

Material and Methods: After Institutional Review Board Approval 271 patients undergoing elective cardiac operation were enrolled into the study between November 20, 2006 and November 19, 2007. Preoperative anxiety was measured by Spielberger State Trait Inventory test, depression was tested by Beck Depression Inventory (BDI) test. Somatic severity score, self-rated health were recorded beside clinical risk factors, EuroSCORE and perioperative characteristics. End points were death and length of stay at the intensive care unit ≥3 days.

Results: Four patients (1.5%) died and 36 patients (13.3%) were ≥3 days at the ICU days. EuroSCORE (3.8±2.8 vs. 4.9±2.9; p=0.026) and somatic severity score (7.8±5.4 vs. 10.3±6.3; p=0.038) were significantly different in patients who needed ICU stay ≥3 days compared to patients who stayed less. Independent predictors of prolonged intensive care were EuroSCORE [1.14 (1.01-1.28), p=0.041], somatic severity [1.08 (1.01-1.15). p=0.020] and BDI score [1.06 (1.00-1.12), p=0.044]. C index of the multivariate model was higher, when psychosocial factors were included (AUC: 0.70) compared to the model of clinical factors only (AUC: 0.61).

Conclusion: Length of ICU after cardiac surgery is influenced by preoperative psychosocial factors, like depression or somatic severity score.

CARDIOVASCULAR

Activation of Cardiac Potassium Channel HERG May be a Determinant of Extracellular Potassium Dependency of Block by Terfenadine

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Introduction: One form of Long QT syndrome referred to as acquired Long QT syndrome has been shown to primarily result from a reduction in the cardiac potassium channel HERG by a number of pharmaceutical compounds. In some instances Long QT syndrome will degenerate into the potentially lethal arrhythmia torsade de pointes, characterized by a rapid heart rate and compromised cardiac output. Many patients requiring medication present with abnormal serum electrolyte levels due to a variety of conditions including gastrointestinal dysfunction, renal and endocrine disorders, diuretic use, alcoholism and aging. Extracellular electrolytes, in particular extracellular potassium have significant influence on HERG channel behavior and have been shown to alter drug block of HERG. However the mechanisms by which drug block is altered in different extracellular solutions are not well understood.

Material and Methods: We used two electrode voltage clamping of Xenopus oocytes to measure block of both wild type and a HERG mutant (D540K) by terfenadine. cRNA of either WT HERG or the D540K mutant was injected into enzymatically defolliculated oocytes and currents recorded 3-5 days after injection.

Results: We previously reported that block of HERG by terfenadine shows the opposite dependency on extracellular potassium compared to quinidine. HERG block by quinidine is greater in 0 mM K (0K) compared to 20 mM K (20K) whereas block by terfenadine is greater in 20K compared to 0K. In order to determine the mechanism underlying this difference in potassium dependency we measured block by terfenadine of the HERG mutant D540K, an usual mutant which opens with both depolarization and hyperpolarization. Block of D540K by terfenadine showed the opposite dependency on extracellular potassium compared to block of WT HERG by terfenadine. Thus block of D540K by terfenadine is greater in 0K compared to 20K similar to the block of WT HERG by quinidine.

Conclusion: Recent experiments indicate that terfenadine is trapped inside the channel after the channel closes whereas quinidine is not. In addition we have reported that block of HERG by quinidine shows a strong correlation with permeant ion. Together these results suggest that the permeant ion is not able to destabilize a trapped drug but is able to destabilize a drug that is not trapped and indicate a possible role for the activation gate in determining the extracellular potassium dependency of block of HERG.

CARDIOVASCULAR

Autonomic Nervous System Modulations in Paroxysmal Atrial Fibrillation

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Introduction: Atrial fibrillation (AF) is a major clinical problem involving a growing number of patients with different clinical manifestations and causing a significant social and economic impact. Only a few clinical investigations were made to verify the existence of a link between AF episodes and autonomic nervous system modulations.

Aim: to investigate deviations of autonomic nervous system tonus in patients with AF prior to onset of paroxysm.

Material and Methods: Time-domain statistical heart rate variability analysis used to assess autonomic nervous system modulation in 24-hour ECG of 28 healthy subjects and 21 patients with paroxysmal AF. RMSSD (the square root of mean of the sum of squares of differences between adjacent NN intervals) parameter is known to reflect both sympathetic and vagal activity. RMSSD values in patients with AF for the last 60 minutes prior to onset of paroxysm were compared with healthy subjects values.

Results: Median RMSSD in patients with AF is higher than median RMSSD in healthy subjects ($p<0,01$). Detailed analysis showed that patients with AF may be divided into two groups: the first group had increase in RMSSD values, the second – decrease. All patients with AF showed RMSSD increase in the last 10 minutes prior to paroxysm. Patients of the first group showed greater RMSSD increase than patients of the second group.

Conclusion: Onset of AF is associated with modulations in autonomic nervous system. Increase in RMSSD observed in the first group reflects the activation of parasympathetic nervous system – patients have vagal-dependant AF paroxysms. Decrease in RMSSD shows sympathetic activation hence the patients of the second group have sympathetic-dependant AF paroxysms. Further studies are needed to investigate the role of vagal activation in the last 10 minutes in onset of AF paroxysm.

ENDOCRINOLOGY

Pioglitazone Increases Amount of Epicardial Fat in Patients with Type 2 Diabetes Mellitus

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Introduction: Diabetes mellitus is associated with an increased risk on cardiovascular disease and epicardial fat has been proposed as an additional cardiovascular risk factor. Treatment with pioglitazone leads to an improvement in glycemic control, but also increases body weight.

The primary aim was to evaluate the effect of pioglitazone on epicardial fat accumulation in patients with type 2 diabetes mellitus (DM2). Yet there is no golden standard to measure epicardial fat. Therefore we developed a new and reliable method to accurately quantify epicardial fat. The secondary aim was to assess the relationship of epicardial fat to anthropometric measurements and fat distribution in these patients.

Material and Methods: Seventy-seven male patients with DM2 were included in this study (mean± SEM, age 56.5 ± 0.6 yr; HbA1c 7.1 ± 0.1%), without cardiac ischemia. Patients were randomly assigned to pioglitazone (30mg/day) or metformin (2000mg/day) and matching placebo during 24 weeks. Epicardial fat was measured by magnetic resonance imaging (MRI) scans in the horizontal and left vertical long-axis plane. Visceral and abdominal subcutaneous fat were measured by MRI at the L4-L5 level. Myocardial and hepatic triglyceride content (TG) were determined by 1H magnetic resonance spectroscopy.

Results: Epicardial fat correlated with abdominal visceral fat ($r = 0.53$, $p < 0.001$),
BMI ($r = 0.42$, $p < 0.001$), abdominal subcutaneous fat ($r = 0.35$, $p = 0.002$), myocardial TG ($r = 0.24$, $p = 0.04$) and hepatic TG ($r = 0.29$, $P = 0.01$).

Both treatments improved glycemic control similarly. Patients treated with pioglitazone had an increased body weight ($p < 0.001$). In this group the amount of subcutaneous adipose tissue increased by 18 % ($P < 0.001$) and total ventricular epicardial fat volume increased by 9% ($P = 0.01$). Also, the fat thickness, measured with different lines, increased at the right ventricle by 16% ($P = 0.040$), but remained the same at the level of the left ventricle. Metformin did not affect these fat compartments. In contrast, hepatic TG content significantly decreased in the pioglitazone group. Abdominal visceral fat did not change in both groups.

Conclusion: Pioglitazone increases right ventricular epicardial fat in type 2 DM patients, whereas it does not affect visceral fat and EAT at the left ventricle. Moreover, the medicine increases body weight, BMI and subcutaneous fat. Furthermore pioglitazone decreases the hepatic TG content.
There is a correlation between EAT and BMI, body weight, abdominal subcutaneous and visceral fat in type 2 DM patients.

ENDOCRINOLOGY

Comparable Diagnostic Performance of the 1 µg ACTH Test and the 250 µg ACTH Test for the Diagnosis of Adrenal Insufficiency

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Introduction: For assessment of adrenal secretory reserve, the 1 µg and 250 µg ACTH tests are available.
Main aim of this study was to compare the diagnostic performance of the 1 µg and 250 µg ACTH test in a large cohort of patients.

Material and Methods: Diagnostic, single center study including all consecutive patients suspected of possible primary or secondary adrenal insufficiency between January 2004 and December 2007 who had both ACTH tests (1 µg and 250 µg) (N=209). A Bland Altman plot with limits of agreement was made to compare these two tests. In a subpopulation (N=55), the cortisol responses in the two ACTH tests (cortisol response 30 min after administration of ACTH) were compared to the maximal cortisol response during an insulin tolerance test (ITT).

Results: The mean difference between the cortisol responses in the two ACTH tests was only 0.03 µmol/l (95% CI 0.015-0.042). The diagnostic performances of the two tests were not different (p=0.49) using a cut-off value of 0.55 µmol/L. The limits of agreement ranged from -0.17 to 0.22. The sensitivity and specificity for the 1 µg test were 0.93 and 0.67, respectively. For the 250 µg test 0.88 and 0.53, respectively. The mean difference between the response of the 1ug test and the ITT was -0.003 µmol/l (95% CI -0.03-0.03). The mean difference between the response of the 250 µg test and the ITT was -0.03 µmol/l (95%CI -0.07-0.07).

Conclusion: This study showed that the 1µg and the 250 µg ACTH test have comparable diagnostic performances in patients with suspected adrenal insufficiency, because of a minimal difference in mean outcome. Compared to the ITT, the 1 µg test seems to be slightly better than the 250 µg ACTH test.

ENDOCRINOLOGY

State of Antioxidant System in Liver of Rats in the Condition of Experimental Diabetes

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Introduction: Diabetes is one of the most widespread diseases. Metabolic disbalance, which arises up from diabetes, is the basic reason in developing of oxidation stress., that leads to increase in processes of lipoperoxidation and antioxidant defense.

Material and Methods: Ten Wistar male rats weighting 180-200 grams have been investigated. Diabetes mellitus was modeled by introducing streptosotocin (50mg/kg of total body weight). The samples of homogenate were taken on 14th day of development of the pathological process, after the induction. The level of hyperglycemia was the criterion of development of disease (18-22 μmole/l), comparing with a control group – 7,2-8,4 μmole/l. made of 10 rats. We estimated nitrogen oxide (NO) according to the Green L.C.,David A.W. method(1982); Superoxide dismutase (SOD) according to Sandstrom J., Nilsson P., Karlsson R. method1994; oxidative modification of proteins(OMP) according to the E.E. Doubininoi method (2000).

Results: The research results show the increase of nitrogen oxide (NO) production in streptosotocin diabetes to 0,751±0,046 mcmol/mg protein in the group of animals with diabetes (control 0,485±0,029 mcmol/mg protein) in the homogenate. The OMP level of carbonil groups increased to 42,5±5,4 OOG/mg protein in the group of animals with diabetes (control 7,2±1,9 OOG/mg protein), and OMP level of basic groups increased to 20,3±3,2 OOG/mg protein in the group of animals with diabetes (control 1,2±0,76 OOG/mg protein). SOD activity was increased to 146,03±11,3 mcmol NST/hv mg protein in the group of animals with diabetes (control 88,82±9,4 mcmol NST/hv mg protein).

Conclusion: The activity of oxidative modification of proteins and antioxidant defence was increased as a result of passing oxidation stress. But level of stable metabolite of nitrogen was rised, that shows on inability of the antioxidant system in a complete measure to counteract the active radicals, which are producted in excessive quantitavely in diabetes.

ENDOCRINOLOGY

Altered Expression of Novel Components of Renin-Angiotensin System During the Development of Spontaneous Hypertension

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Introduction: Renin-angiotensin system(RAS), the principal regulator of blood pressure, may be involved in the development of essential hypertension. Angiotensin converting enzyme (ACE), angiotensin II (AngII) and its receptor (AT1) represent a well-known axis of RAS. The activation of this axis leads to vasoconstriction and increase in blood pressure. Novel local components of RAS were discovered recently, including angiotensin converting enzyme 2 (ACE2) converting AngII into angiotensin(1-7). Ang(1-7) induces beneficial vasodilatory and antiproliferative effects through activation of Mas receptor. Moreover, Mas receptor interferes with AT1 receptor preventing its activation by Ang II. ACE2/Ang(1-7)/Mas forms a novel RAS axis balancing the activity of ACE/AngII/AT1 pathway in the regulation of blood pressure. We have hypothesized that changes in the expression of ACE, AT1, ACE2 and Mas receptor might be involved in the development of spontaneous hypertension. Additionally, we have examined whether perinatal AT1 receptor blockade with losartan may modulate these expressions.

Material and Methods: Blood pressure was measured by tail-cuff method in Wistar Kyoto rats (WKY, n=5), SHR (n=5) and SHR treated with losartan (20 mg/kg/day, p.o. twice daily) perinatally up to 9 weeks of age (n=5, dams during gestation/lactation period and pups from weaning at 4 weeks). At the age of 9 weeks, ACE, ACE2, AT1 receptor and Mas receptor mRNA levels in renal cortex and left ventricles were determined by Real-Time PCR.

Results and Conclusion: Perinatal losartan treatment (105±3 mmHg) prevented the rise in blood pressure (109±2 vs. 149±2 mmHg in WKY and SHRs, respectively). Compared to WKY, renal expression of ACE was unchanged in SHRs, whereas expression of AT1 receptor was significantly lowered. Renal expression of ACE2 was upregulated more than two-fold in SHR rats, while the expression of Mas receptor was substantially reduced. On the contrary, in the heart of SHRs, ACE expression was reduced when compared to WKY, while the AT1 receptor expression remained unchanged. In SHRs, the cardiac expression of ACE2 was not changed, but Mas expression was decreased. Despite of the modulation of blood pressure, losartan failed to affect ACE, AT1 and Mas expressions while it upregulated ACE2 in the kidneys of SHRs. Our results suggest that local RAS is regulated differentially in the kidney and the heart of SHRs. Downregulation of ACE, AT1 receptor and upregulation of ACE2 in SHR rats may represent mechanisms compensating to elevated blood pressure. However, lack of expression of Mas receptor may be involved in the development of spontaneous hypertension.

GYNAECOLOGY

Prevalence and Age Distribution of Human Papillomavirus among Females in Region of Tuzla, Bosnia and Herzegovina

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Introduction: A human papillomavirus (HPV) is a papillomavirus that induces hyperproliferative lesions in cutaneous and mucosal epithelia. So far more than 100 HPV genotypes have been identified and associated with cancerous and precancerous lesions of the cervix. Certain types, such as HPV 16 and 18 are highly carcinogenic, while others, such as HPV 31 and 33 are moderate. Types, such as HPV 6 and 11 are mildly carcinogenic. Worldwide, right after breast cancer, cervical cancer is the second most common cancer that affects women. The aim of this study was to determinate the prevalence and age distribution of HPV infections among females in the Tuzla Canton between years 2000 and 2008, and to reveal the type of cytological change found in women born in 1971 and later.

Material and Methods: This study has been conducted by a retrospective analysis, using the information from Policlinic for Laboratory Diagnostics-Department of Pathology, University Clinical Center Tuzla. Samples of the cervical smear for the PAP test and HPV analysis were taken during routine gineacological examinations, by using brush and sticks with cotton, taken from the Digene Specimen Collection Kit, from the whole surface of a portion, and by mild rotating moves from the outer cervical entrance. Identification of the presence of HPV was carried out by the Digene Hybride Capture II test in all the cases. The cytomorphological changes found using light-microscope method were classified according to the Bethesda classification of cervical lesion.

Results: From January 2000 until April 2008, a total of 4918 women were recruited into the study and had their Pap smear tested. Positive Pap smear was found in 2091(42,5%) women of all ages. Our results showed that the most tested group was born between 1961 and 1970 (29.28%), followed by women born in 1971 and later (28.54%). The most frequent abnormal PAP test at HRHPV+ women born in 1971 and later was LGSIL (40,09%) followed by CC (30,91%) and HGSIL (28,98%).

Conclusion: HPV is very common among females in the region of Tuzla. Our data indicates an increased number of tested women in all age groups after a conducted media campaign. Our study does not coincide with other studies that were done in USA. The most tested and affected group of the women in our study were those born between years 1961 and 1970.

GYNAECOLOGY

New Applications for Neonatal and Prenatal Screening / Diagnostics of the Hemoglobinopathies

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Introduction:Sickle-cell disease (SCD) and beta-thalassemia major are two common forms of hemoglobinopathy with severe outcome. Children with SCD develop severe painful crises due to multiple infarctions, tissue degeneration and organ damage while children with beta-thalassemia major become transfusion dependent and remain as such life long. In the Netherlands, neonatal screening (NS) for hemoglobinopathies has started in January 1st 2007 using state of the art HPLC technology.

Material and Methods: Screening of 1500 anonymous cord blood samples using the capillary electrophoresis (CE) technology (Sebia, France). Identification of carriers will be confirmed according to published DNA methods. Exploration of the possibility to detect beta-thalassemia carriers (not detected so far) using accurate HbA measurement. Current and new technologies will be tested for their diagnostic application in regular and non-invasive prenatal diagnosis.

Results: We are presenting the validation of an alternative method to identify carriers of SCD by the regular separation of HbS, C, E and D on CE. Moreover, we report sensitivity data on a selective method to identify carriers of beta-thalassemia trait by the levels of hemoglobin A_2 present at birth.

Conclusion: Without preventive measures 60 new cases can be expected yearly in the Netherlands. The CE technology is a sensitive alternative to HPLC for the screening of hemoglobinopathies. Identification of neonates-carriers of SCD and beta-thalassemia based on biochemical techniques facilitates prevention of beta-thalassemia major due to combinations of beta-thalassemia and HbS.

GYNAECOLOGY

Subclinically Increased Troponin T levels in Preeclamptic Patients are Correlated with the Development of Pulmonary Edema

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Introduction: Pulmonary edema is a serious complication in preeclampsia. Cardiac failure is considered as a possible cause of this complication. Preliminary reports describe subclinically increased Troponin T (TnT) levels, a marker of myocardial damage, in patients with hypertensive disorders of pregnancy. In this present study, the relation of TnT levels and the development of pulmonary edema in preeclamptic patients was examined.

Materials & Methods: A prospective, longitudinal survey was conducted using medical files. From September 2004 until December 2005 all patients diagnosed with preeclampsia (mild or severe) were reviewed. Pulmonary edema was defined using common clinical parameters: clinical dyspnoea, crepitating upon auscultation, oxygen saturation < 95%, arterial oxygen content < 10 kPa, use of supplemental oxygen, administration of diuretics, ct-scan or chest X-ray with signs of pulmonary edema. During admittance laboratory tests including TnT levels were repeated at least twice weekly in mild preeclampsia and daily in cases of severe preeclampsia. TnT levels were regarded positive at ≥ 0.03 μg/ L. Comparisons of clinical parameters between mild or severe preeclamptic patients were made using Mann-Whitney or Fisher's exact test. Conditional logistic regression analysis, allowing for the daily TnT positivity as a time-dependent variable, was used to evaluate associations with the occurrence of pulmonary edema.

Results: During the study period, 219 patients were admitted with the diagnosis preeclampsia. In 138 patients, 92 patients with mild preeclampsia and 46 patients with severe preeclampsia, at least one TnT measurement was available. Ten patients developed pulmonary edema within two days after delivery. None of the patients developed pulmonary edema before delivery. During these days, at least one TnT measurement was available in 122 patients. Sixteen patients had no TnT measurements, these patients were excluded for further analysis. Combining the data of the 122 patients using Cox regression, preeclamptic women with subclinically increased TnT levels were more likely to have or to develop pulmonary edema (OR = 18.1, 95% CI 3.1-103.2, P=0.001).

Multivariable analysis, adjusted for severity of preeclampsia, revealed that patients with severe preeclampsia were more likely to develop pulmonary edema than those with mild preeclampsia (OR = 6.1, 95% CI: 1.2-61.0, P=0.03). Subsequently, severe preeclamptic patients with elevated TnT levels were more likely to develop pulmonary edema (OR = 16.3, 95% CI: 2.5-106.9, P=0.003).

Conclusion: Subclinically increased TnT levels are strongly correlated with the development of pulmonary edema in patients with predominantly severe preeclampsia. Myocardial failure is a possible cause of pulmonary edema in preeclamptic patients.

GYNAECOLOGY

Changes in the Endometrial Receptivity in Tubular Infertility: Morphological Study

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Introduction: Functional salpinxes are one of the most important preconditions for female fertility. Tubular infertility - TFI (Tubular Factor Infertility) is the most significant of salpingeal pathologies, resulting in uni- or bilateral partial or complete salpingeal occlusion. Tubular infertility is one of the leading causes of infertility in the world including Estonia. The most common method treating infertility due to TFI is In Vitro Fertilization (IVF). Occurrence of TFI decreases receptivity of the uterine mucosa, lowering the expression of αvβ3 integrins on the endometrial endothelium and therefore lowers the possibility of implantation. Noticeable changes in cytokine profile (higher levels of TNF-α) of the uterine endothelium in tubular infertility have also been reported, influencing uterine maturation. For the interaction between the endometrium and the embryonic cells special structures called pinopodes are needed. Pinopodes can be studied using electron microscopy. The role of pinopodes in TFI and infertility has not been thoroughly studied yet. Our study centers on finding out the causes of tubular infertility.

Material and Methods: Ten endometrial biopsies of patients with infertility problems were carried out in Nova Vita Clinic. Taken tissue samples were divided into two. Half of them were fixed in 10% buffered formalin solution and the other half in 2.5% glutaraldehyde solution. Sections embedded in paraffin were studied with light microscopy. For electron microscopic assessment sections were embedded in plastic (Epon 812) according to classical methods. Histological methods were used to specify the stage of menstrual cycle. Immunohistochemical staining was used to detect integrin beta3 (CD61) in the endometrium. Scanning electron microscopy (SEM) was used to assess endometrial glands, endometrial epithelial cells and pinopodes.

Results: Light microscopy studies of biopsies revealed no pathologic changes in endometric morphology. The ratio between glandular and stromal components was 1:1 in five samples. In one sample corresponding ratio was 2:1. The sample also showed lots of spiral arteries. Immunohistochemical staining with CD61 showed a very intense reaction in one patient, mild reaction in two patients and low reaction in seven patients. For SEM assessment only 5 samples were suitable. In one patient SEM showed normal endometrial epithelial cells. All the other patients showed morphological changes in ciliated epithelial cells. Pinopodes, essential components for embryonic implantation, were present in only one of the patients samples.

Conclusion: SEM studies have shown that TFI patients have appreciably lower amount of pinopodes needed for the implantation of embryo

HAEMATOLOGY

Diagnostic Efficacy and Safety of Computed Tomography-Guided Transthoracic Needle Biopsy in Patients with Hematologic Malignancies

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Introduction: The role of transthoracic needle biopsy (TTNB) in patients with hematologic malignancies, particularly discriminating between malignant and benign etiologies, has not been well studied. Hence, a retrospective analysis to evaluate the diagnostic efficacy and safety of computed tomography (CT)-guided TTNB in this population was performed.

Material and Methods: Records of 54 patients with hematologic malignancies, who underwent TTNB from August 1, 1999 to July 31, 2007, were retrospectively reviewed and analyzed. Particular consideration was given to changes in antifungal therapy based on results of TTNB.

Results: The most common underlying hematologic malignancy was non-Hodgkin lymphoma in 21 patients (38.9%). Of lesions biopsied, the most common lesion location was the left upper lobe in 16 cases (29.6%); 33 lesions were pleural-based (62.3%), and 9 had cavitation (16.7%). Samples were considered cytopathologically adequate in 24 cases (48%). TTNB established a specific diagnosis in 24 patients (44.4%): malignancy was found in 12 (22.2%), infection in 10 (18.5%), and a non-specific benign diagnosis in 2 (3.7%). Sensitivity for detecting malignancy was 47.8%, and sensitivity for detection of a specific infection was 38.5%. Complications occurred in 9 patients (16.7%), pneumothorax in 8 cases (14.8%) and self-limited small volume haemoptysis in 1 case (1.9%). One patient (1.9%) required chest tube placement. The results of TTNB led to a change in antifungal therapy in 14 of the 24 patients with a specific diagnosis (58.3%).

Conclusion: Optimized TTNB in this population is a safe and efficacious diagnostic test, and has decreased morbidity and mortality compared with surgical procedures

Conclusion:

1. Patients with anterior MI usually have worse outcome and prognosis compared with those with inferior (posterior) MI.
2. Men with AMI develop complications more often than in woman (ratio 2:1).
3. Appearance of complications in patients with AMI is connected with systolic dysfunction of the left ventricle.

HAEMATOLOGY

Variations of Iron Metabolic Indexes in Megalobalstic Anemia

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Introduction: Iron metabolic indexes, including serum iron (SI), total iron binding capacity (TIBC) and serum ferritin change in hematologic disorders. TIBC increases in iron deficiency or pregnancy, and decreases in hemolytic anemia and anemia of chronic diseases .also ferritin increases in hemolytic anemia, leukemia and inflammatory disease and decreases in iron deficiency or pregnancy. We studied the change of these indexes in megalobalstic anemia patients.

Material and Methods: By reviewing the files of the patients admitted in UEKEH, with diagnosis of M.A and we charted the range of S.I, serum ferritin and TIBC.

Results: Out of 135 patients with M.A- 46% female and 54% male in the range of 15-95 years old with the average of 52.9- we found mentioned lab tests in 104 of them and the average of 110 for SI, 251 for TIBC and 245.4 for serum ferritin.

Conclusion: This study indicates that the change of iron metabolism indexes in M.A is similar to their range in inflammatory diseases, therefore, in patients with these range of iron indexes and without any significant signs or symptoms of inflammation - specially for the cases without macrocytosis such as minor thalassemia - we have to think of M.A beside hemolytic anemia as a differential diagnose.

HAEMATOLOGY

Cardio Toxic Effects of Anthracycline Therapy in Children with Acute Lymphocyte Leukemia

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Introduction: Over the last 25 years of clinical trials, a significant rise in the rate of complete remissions as well as an increase in long-term survival of children with acute lymphoblastic leukemia has been achieved. Therefore, growing attention is now focused on the toxic effects of chemotherapy.
Cardio toxicity is well known side effect of chemotherapy with antracyclins.

The aim of our study was to evaluate the cardio toxic effects of chemotherapy based on the EF (Ejection fraction), FS (Shortening fraction) and tachycardia.

It is retrospective study of 60 patient records with ALL in the period July 2001-June 2007. This study is a part of a bigger study that involves all toxic effects of the ALL-BFM-95 protocol.

Material and methods: Evaluation of ECG and cardiac echosonography in children who manifested cardio toxicity during the chemotherapy. We evaluated the ones made before starting the protocol and further ECG (made weekly) and echo sonograms made after indication of cardiologist. We evaluated Ef (Ejection fraction), Fs (Shortening fraction) and changes in the rhythm.

Results: From the total number of 60 patients, 21 manifested cardiotoxic effects. Fifteen were female and six male (2,5:1), and the average age was 7 years, (1.5 - 12 years). The changes that were registrated were: tachycardia in 19 cases (90%), frontal pericardial separation in 2 cases, problems with repolarization in 5 patients (23.8%), 3 cases of initial secondary cardiomyopathy (14.3%) and 1 case of cardiac hypertrophy(4.76%). All of the changes disappeared after the end of the chemotherapy, except the tachycardia. Tachycardia manifested 10 patients in protocol I, after the cumulative dosage of 120 mg/m² of anthracyclines, in protocol M manifested 4 patients, and 5 patients in protocol II after cumulative dosage of 240 mg/m². Patients were treated with Digoxin and at the end of the treatment they were recommended to continue Digoxin therapy and to do regular checkup at the cardiology department. The EF was in the referent values of 65-83 in all of the patients. It was similar with the FS; there were no significant changes in the values.

Conclusion: The major problem was sinus tachycardia, a disorder of the rhythm in 90% of the children. Several children manifested reversible changes in echocardiogram. None of them had significant changes in EF and FS. Further follow-up of these patients is necessary to detect eventual late cardio toxic effects of chemotherapy.

HAEMATOLOGY

Correlation Between Cerebrospinal Fluid Tau Protein and Cognitive Functioning in Children with Acute Lymphoblastic Leukemia

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Introduction: Event free survival in childhood acute lymphoblastic leukaemia (ALL) rose above 70%, allowing us to observe a variety of long-term complications, including neurological disorders. After replacing central nervous system (CNS) radiotherapy and systemic plus intrathecal chemotherapy with high-dose of methotrexate, number of neurological disorders has decreased. However decline in neurocognitive functions can occur even years after the treatment. The aim of the study was to assess whether cerebrospinal fluid tau protein is associated with cognitive changes in children with ALL.

Material and methods: We have examined 38 patients (22 boys, mean age at the diagnosis 7.59 years) with ALL from Department of Pediatric Oncology and HAEMATOLOGY of Medical University in Bialystok, Poland. Cerebrospinal fluid (CSF) samples were collected from children during lumbar punctures performed at diagnosis (point 1), after induction treatment [protocol I (point 2)], during consolidation [protocol M (points 3, 4, 5, 6)] and before maintenance therapy [protocol II or protocol III (point 7)]. The reference group consisted of 22 children (12 boys, mean age 8.23), diagnosed with the clinical symptoms of cerebrospinal meningitis. The Human Total Tau Enzyme-Linked Immunosorbent Assay Kit (BIOSOURCE) was used to determine tau protein levels. Cognitive functioning was established in 19 patients, on average 3.7 years after diagnosis, using Wechsler' Intelligence Scale for Children-Revised (WISC-R). Data were analysed with statistical package (Statistica 6.0).

Results: The total protein level in the cerebrospinal fluid was not elevated in any of the samples. The mean value of tau protein at diagnosis was 286.81+/-121.26 pg/ml in the study group and 297.63+/-96.81 pg/ml in the reference group. Age and gender did not influence the level of tau protein in CSF of both group. Examination revealed a statistically significant increase in tau protein on the 59-th day of the treatment and at two points during consolidation phase. The level of tau protein at point 7 (before maintenance therapy) was negatively correlated with verbal intelligence quotient measured on an intellectual scale ($r=-0.65$ $p<0.006$).

Conclusion: It seems that raised level of CSF tau protein during intensive chemotherapy is indicative of functional abnormalities and cell injury of CNS. The study suggests that standard ALL treatment may cause decline in cognitive functioning.

HAEMATOLOGY

Effect of the Selective Serotonin Reuptake Inhibitor Paroxetine on Platelet Function is Modified by a SLC6A4 Serotonin Transporter

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Introduction: Selective serotonin reuptake inhibitors (SSRIs) have been associated with an increased bleeding tendency. Polymorphisms of the serotonin transporter (SERT) gene (SLC6A4) promoter region (5-HTTLPR) are associated with the transcriptional activity of the SERT-gene and the rate of serotonin uptake. The objective of this study was to prospectively quantify the dose-response effects of paroxetine and the influence of the 5-HTTLPR polymorphism on platelet function.

Material and Methods: Nineteen drug-free psychiatric outpatients with anxiety disorder or depression (44.5 +/-10.8 years), were tested before and after 6 weeks of paroxetine treatment (20mg/day). Based on clinical symptoms, paroxetine dosages were increased (40-50mg/day) for 6 more weeks in 11 patients. Parameters related to platelet function were assessed by bleeding time, platelet function analyzer (PFA), platelet serotonin, platelet factor 4 (PF4), beta-thromboglobulin (beta-TG), and aggregation tests.

Results: Paroxetine 20mg/day increased mean bleeding time by 1.2 minutes (95% confidence interval (95%CI) -0.2-2.7) and reduced median platelet serotonin level (463 ng/10(9) platelets; Inter Quartile Range (IQR) 361-666), and platelet ss-TG concentration (3.1 IU/10(6) platelets; IQR 0.3-6.0). Other platelet parameters did not change significantly. Serial platelet aggregation tests did not become abnormal. Paroxetine dose-escalation did not further influence platelet function. However, 5-HTTLPR polymorphisms modified these effects: in L(A)/L(A)-carriers, bleeding times did not change (-0.2 minutes; (95%CI -0.6 to 0.9)), while bleeding times significantly increased in <2L(A)-allele carriers (2.3 minutes (95%CI 0.5 to 4.07); p=0.032). Platelet serotonin decreases were larger in patients without L(A)-alleles (868 ng/10(9) platelets; (IQR 585 to 1213)) than in >/=1 L(A)-allele carriers (457 ng/10(9) platelets; (IQR 392 to 598); p=0.035). PFA closure time and PF4 increased significantly in patients without L(A)-alleles.

Conclusion: Paroxetine 20mg/day does not increase overall bleeding time, but impairs platelet function by decreasing the levels of platelet serotonin and platelet ss-TG. These paroxetine effects appear to be mediated by 5-HTTLPR, with most pronounced effects in patients without L(A)-alleles.

HAEMATOLOGY

Characterization of a Novel Fusion Gene Possibly Involved in the Development of T-cell Acute Lymphoblastic Leukemia

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Introduction: T-ALL is a T-cell malignancy affecting children and adolescents, caused by cooperation of mutations affecting proliferation, survival, cell cycle, and differentiation.

To detect novel genomic rearrangements, we performed 1 Mb resolution array CGH with extra probes covering candidate oncogenes, as the RTKs (Receptor Tyrosine Kinase). A new fusion gene involving an RTK which functions by kinase activity and nuclear translocation, was detected in one cell line. The other gene involved is a transcription factor expressed in lymphocytes that regulates development and differentiation. Specific screening for this fusion transcript showed it's presence in 31% in a higher number of T-ALL cell lines.

Materials and Methods: constructions: we generated the sequence of the different fusion variants found in the cell lines by PCR followed by clonation into the MSCV-puromycin vector. Transfection of Hek293T cells was done with Genejuice reagent and the expression and activity level of the RTK was analyzed by Western Blotting. Screening in patients: Samples from 25 patients with T-ALL were screened for the presence of the fusion transcript at RNA level. Cell culture: T-ALL cell lines were cultured in RPMI-1640 supplemented with 20% FCS. We cultured BaF3 cells in RPMI-1640 medium supplemented with 10 % FBS and 1 ng/ml mouse IL3.By retroviral transduction and subsequent puromycin selection we made BaF3 cells express the different constructs. To test IL3-independency, stable BaF3 cell lines were washed three times in PBS and cultured in IL3-free medium. The number of viable cells was counted with a Vi-cell XR cell viability analyzer (Beckman Coulter, Fullerton, CA).
Results: The new fusion transcript was detected in 16% (4/25) of the patients.

Transduction of the constructs in BaF3 cells revealed that none had transforming capacities. Overexpression of the wild type RTK could make the cells IL3 independent after stimulation of the kinase by its ligand.

Conclusion: The presence of a fusion transcript in 31% of the cell lines and 16% of patients with T-ALL suggests a possible role in the development of leukemia. The observation that the addition of the ligand to cells overexpressing the RTK made them IL3 independent, confirms that this kinase can transform thymocytes. Functional studies are still in progress.

IMMUNOLOGY

The Influence of Trappin-2 on the Phagocytic Capacity of Macrophages

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Introduction: Trappin-2 is an inhibitor of human neutrophil elastase with antimicrobial properties present in the lung. Previous research has shown a protective effect of trappin-2 in animal models of pulmonary infection, due to various mechanisms. This study aimed to investigate whether trappin-2 could also enhance phagocytosis of Pseudomonas aeruginosa by macrophages.

Material and Methods: The effect of trappin-2 on clearance of P. Aeruginosa was assessed by in vitro macrophage antibiotic assays using murine bone-marrow derived macrophages, MH-S murine alveolar macrophages and human alveolar macrophages by co-incubation with 100 nM trappin-2 and P. Aeruginosa.

Results: Macrophage antibiotic assays using murine bone-marrow derived macrophages and human alveolar macrophages did not result in significant differences between treatments. In experiments using murine alveolar macrophages, pre-incubating P. Aeruginosa with trappin-2 resulted in a non-significant but consistent trend towards a synergistic effect of both trappin-2 and murine alveolar macrophages on bacterial clearance. Incubating bacteria with trappin-2 and macrophages resulted in a decrease of bacteria of 43.6% compared to macrophages alone whereas adding trappin-2 to bacteria without macrophages showed a 23.1% reduction in bacteria ($p>0.05$).

Conclusion: If reproducible in repeat experiments, these data support the concept that trappin-2 could act as an opsonin and therefore be a target for new therapeutic strategies in pulmonary infection.

IMMUNOLOGY

Marked Differences in Fine Specificity and Isotype Usage of the Anti-Citrullinated Protein Antibody in Health and Disease

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Introduction: Anti–citrullinated protein antibodies (ACPAs) display high association with rheumatoid arthritis (RA) and are implicated in its pathogenesis. The presence of ACPAs is known to precede the onset of RA. In order to identify the features that could confer its pathogenicity, we extensively characterized this antibody response in a unique North American native population of patients with RA and their unaffected relatives.

Material and Methods: The levels of IgA, IgM, and IgG ACPAs, as well as IgM and IgA rheumatoid factor (RF), were measured in serum samples obtained from 81 patients with RA and 195 of their unaffected relatives. The isotype distribution, the fine specificity of the ACPA response, and its association with RF were compared in health and disease.

Results: ACPA positivity was observed in 19% of the healthy relatives and 91% of the patients with RA. ACPA isotype usage was strikingly lower in unaffected relatives than in patients with RA (1–2 versus 5–6 isotypes). Fine specificity studies showed that reactivity to citrullinated fibrinogen and vimentin was present in sera from patients with RA, while it was virtually absent in their unaffected relatives. Finally, the ACPA and RF responses were associated in patients with RA but were discordant in their healthy relatives. Extended analyses revealed that the presence of ACPAs was associated with RA irrespective of RF status, while the association of RF with disease relied on its interaction with ACPAs.

Conclusion: The fine specificity and isotype usage of the ACPA response are qualitatively different in health and disease. Epitope spreading and expansion of the isotype repertoire might be necessary for development of RA, and this could be facilitatedby the presence of RF antibodies.

IMMUNOLOGY

The Artificial Thymus

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Introduction: The thymus is an organ that produces T cells for immunity. However, sometimes the thymus does not work properly, or is completely absent. In case of autoimmune diseases or immunodeficiency, treatment could consist of implanting an artificial thymus, that produces new T cells, makes new immunity, or stops the auto-immune reactivity. An artificial thymus could also be a possible treatment for cancer, with the purpose of producing new T cells that can attack cancer. Therefore, the aim of the present research was to construct an artificial thymus.

Material and Methods: For the production of an artificial thymus, stromal cells (for example thymic epithelial cells) are required, and therefore a mesenchymal cell-line was used, TST-4/DLL1.
It has been shown before that in the development of an artificial lymph node in vivo, a collagen sponge works as an efficient scaffold. In the present study we tested whether a collagen sponge was also suitable for the formation of an artificial thymus in vivo. Also we tested a re-aggregate artificial thymus. All artificial thymuses were transplanted in mice.

Results: We found that the collagen sponge can support T cell development, but not very efficiently. A re-aggregate lobe however, is more efficient to construct an artificial thymus. We found that a re-aggregate lobe of TST-4/DLL1 could restore T cell differentiation in nude mice, lacking the thymus. Transplantation of this re-aggregate lobe under the kidney capsule of this nude mice resulted after 14 days in the presence of mature T cells.

Conclusion: It is possible to construct an artificial thymus, however more research is needed to prolonging the effectiveness of the artificial thymus. We hope we have taken some first steps for a new treatment for auto-immune diseases and maybe for cancer and HIV.

IMMUNOLOGY

Analysis of High Resolution Computed Tomography Findings in Patients Suffering from Lung Sarcoidosis

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Introduction: Sarcoidosis is a multisystemic, noncaseating granulomatous disease of unknown origin. Most commonly involved are the lung parenchyma, pleura, and hilar lymph nodes.
Aim: The aim of this research was the analysis of high resolution computed tomography (HRCT) results from patients suffering from lung sarcoidosis.

Material and Methods: Disease histories, CT and HRCT from the electronic archives of patients suffering from lung sarcoidosis were processed in a retrospective study. The study involved 52 patients (aged 45,3 +/- 10,9 years), 34 (65,38%) female gender (aged 47,4 +/- 10,8 years) and 18 (34,62%) male gender (aged 41,4 +/- 10,5 years). During the course of this study the analysis was made concerning presence and frequentness CT and HRCT signes characteristic for lung sarcoidosis, also concerning their distribution in the craniocaudal (CC) and centro-peripheral (CP) direction, and concerning the symmetry of the lesions.

Results: The characteristical CT findings of bilateral hilar and paratracheal swelling of lymph nods, without the presence of changes in the lung parenchyma was found in 33 (63,46%) patients. Typical diffuse reticulonodular lesions in the interstitium of the lung were found in 43 (82,69%) patients. Definitive lesions (individual cysts, multiple cysts, tractional bronchiectasias, conglomerate masses of fibrous tissue and dislocation of the bronchus), cosequences of advanced fibrosis, were found in 15 (28,85%) patients. Craniocaudal predominance (registered with 19 (36,54%) of patients) and symmetry of lesions (found in 35 (67,31%) examined cases) are a common finding in patients suffering from lung sacoidosis. The centro-periferal predominance on the distribution of lesions is an extremely rear radiological picture in lung sarcoidosis patients (found in 2 (3,85%) patients), as are excavated lesions and pleural effusions (not found in any of the examined cases).

Conclusion: High resolution computed tomography has a great siginficance in diagnosing sarcoidosis because it is a more sensitive and more specific method then standard radiography of the chest. The primary role of HRCT is to differentiate between patients with typical sarcoidosis lesions and those with other diffuse interstitial lesions.

IMMUNOLOGY

The Role of IL-10 Producing Regulatory B Cells in Protection for Allergic Disease during Chronic Schistosomiasis

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Introduction: Allergic disorders, characterized by allergen-specific T helper type (Th) 2 cell immune responses, are a major health concern in the Western world. Epidemiological studies demonstrated that chronic Th2-skewed helminth infections are negatively associated with allergic disorders and may strongly suppress T cell responses to bystander antigens, such as allergens. The cytokine IL-10 is central to helminth-induced immune regulation of allergic responses and is in part secreted by helminth-induced regulatory T (Treg) cells but may also be produced by a subset of B cells. IL-10 producing B cells have a great potential in regulating T cell mediated inflammatory responses and therefore are named regulatory B cells (Bregs). The role of Bregs has been mainly studied in murine models of hyperinflammatory Th1 responses such as rheumatoid arthritis and experimental autoimmune encephalomyelitis. Several B cell subsets could be candidates to develop and act as regulatory B cell, namely CD5+ B1, follicular (FO), marginal zone (MZ), and transitional B cells.

Material and Methods: We sought to address the question whether IL-10 producing B cells play a role in the helminth-induced immunoregulatory processes during the chronic stage of schistosome infections. Furthermore, we investigated which B cell subset has the capacity to develop into Breg cells. We infected mice with helminth Schistosoma mansoni and sensitized and challenged the mice with ovalbumin (OVA) to induce allergic airway inflammation. To this end, mice were analyzed at 8 or 16 weeks, representing the acute or chronic phase of infection. We purified B cells from the spleen and sorted them into FO and MZ B cells. Next, we investigated the IL-10 production of different B cell subsets, their capacity to induce FoxP3+ Treg cells, and the expression of certain surface markers.

Results: Using IL-10-/- B cell deficient mice, we demonstrated that the helminth-mediated protection against OVA-induced allergic airway inflammation was crucially dependent on the presence of IL-10 producing B cells. Most notably, we detected that MZ B cells were the highest IL-10 producers during chronic schistosomiasis, in particular in response to TLR ligands PGN, Poly I:C, CpG and LPS plus helminth antigens. In addition, MZ B cells also induced the highest percentage of FoxP3+ T cells in vitro and in vivo. Furthermore, we observed that CD5+ B1, FO and MZ B cells were different in their expression of certain surface markers.

Conclusion: Taken together, these findings underline an important role for schistosome-induced regulatory MZ B cells in the protection against allergic airway inflammation.

IMMUNOLOGY

Allergy to Animal Fur and Feathers in Patients with Different Allergic Diseases

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Introduction: Establishing the frequency of allergy to animal fur and feathers in patients with bronchial asthma, seasonal rhinitis and other allergic diseases.

Material and Methods: The research was carried out in 160 patients (105F and 55M) aged 12-64 treated in the Outpatients Clinic of Allergic Diseases. Every patient was interviewed for allergy. Every patient had:
1. Spirometry at rest and bronchodilatatory test
2. Skin-Prick test for inhaled allergens and animal fur and feathers.

Results: In 160 patients we found positive Skin Prick tests for dogs dander in 41 cases (25,6%), cats in 40 cases (25%), hamster in 15 cases (9,3%), horse in 10 cases (6,25%), guinea pig in 8 cases (5%) and feathers in 15 cases (9,3%). Positive result for at least one allergen in 69 cases (43,13%). In the group of patients 25 suffered from seasonal allergic rhinitis, 56 suffered from bronchial asthma, 32 suffered both from asthma and seasonal allergic rhinitis and 47 suffered from other allergy. In 25 patients with seasonal allergic diseases positive Skin Prick test for dogs dander in 5 cases (20%), cats in 5 cases (20%), hamster in 2 cases (8%), horse in 0 cases (0%), guinea pig in 1 cases (4%) and feathers in 3 cases (12%). In 56 patients with bronchial asthma positive Skin Prick test for dogs dander in 11 cases (19,6%), cats in 9 cases (16,07%), hamster in 5 cases (8,9%), horse in 5 cases (8,9%), guinea pig in 6 cases (10,7%) and feathers in 5 cases (8,9%). In 32 patients with bronchial asthma and seasonal allergic rhinitis positive Skin Prick test for dogs dander in 16 cases (50%), cats in 15 cases (46,9%), hamster in 2 cases (6,25%), horse in 2 cases (6,25%), guinea pig in 0 cases (0%) and feathers in 1 cases (3,125%). In 47 patients with other allergies frequency of allergy to dogs dander in 9 cases (19,15%), cats in 11 cases (23,4%), hamster in 6 cases (12,77%), horse in 3 cases (6,38%), guinea pig in 1 cases (2,13%) and feathers in 6 cases (12,77%).

Conclusion:
1. In the group of patients with bronchial asthma and seasonal allergic rhinitis allergy to dog and cat dander is more frequent then in other groups (50% and 46,9%)
2. Allergy to animals is a huge problem among patients with allergic diseases (43% with positive Skin Prick test for at least one animal allergen).

IMMUNOLOGY

FoxP3+ Regulatory T Cells in Childhood Allergic Rhinitis and Asthma

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Introduction: Regulatory T cells (Tregs) play a central role in the pathogenesis of immune mediated disease. We investigated CD4+FoxP3+ and CD4+CD25high regulatory T cells in children with allergic rhinitis and asthma, and compared their prevalence to that in healthy children. We also aimed to describe the relationship between the number of Tregs and disease severity. The prevalence of activated lymphocytes, the target cells of Tregs was also tested.

Material and Methods: 8 children with allergic rhinitis, 8 with mild asthmatic symptoms and 6 with moderate-severe asthma were enrolled along with 13 healthy age-matched children (median age, range: 9.5 [5-18] years). Children with moderate-severe asthma were also reassessed one month later when their symptoms improved. Peripheral blood samples were taken, and mononuclear cells were isolated. Lymphocytes were then detected with flow cytometry using specific antibodies.

Results: The prevalence of CD4+FoxP3+ cells in patients with allergic rhinitis and mild asthma was comparable to that in healthy controls (median, quartile: 2.54 [1.63-3.56]%; 1.90 [1.07-2.36]%; 1.68 [0.88-3.24]%, respectively), but it was significantly increased in patients with moderate-severe asthma (median, quartile: 4.18 [3.35-6.27]%, $p = 0.027$). Furthermore, our trend analysis revealed an association between disease severity and prevalence of CD4+FoxP3+ cells ($p = 0.01$). In patients with moderate-severe asthma after one month of therapy, FoxP3 values showed a tendency to decrease. The prevalence of activated (CD25+, CD62L+ or HLA-DR+) CD4+ and CD8+ lymphocytes was comparable in patient and control groups. No association between activated lymphocytes and FoxP3 values was revealed.

Conclusion: In this study we found that the prevalence of CD4+FoxP3+ regulatory T cells is increased in children with moderate-severe asthma. The shift of Th1/Th2 ratio to the Th2 direction is a well known phenomenon in allergy. The explanation for this skewness has been unclear. Increased Treg cell function may be a contributing factor, as the inhibitory effect of this cell type is more pronounced on Th1 than on Th2 type CD4+ cells. We could not detect an association between CD4+FoxP3+ and CD4+CD25high cells, which supports the notion that CD25 is rather an activation marker and not a specific marker of Tregs, and no clear distinction can be made between CD25+ and CD25high cells. Hence, the prevalence of CD4+CD25high cells does not necessarily reflect that of Tregs. We also found a positive correlation between increasing Treg prevalence and severity of disease. Therefore our results support the contribution of Tregs to severe asthma in childhood.

IMMUNOLOGY

Aeroallergens in Allergic Rhinitis Patients, in Tehran, Iran (Comparing Adult and Children)

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Introduction: Allergic rhinitis (AR), the most prevalent allergic disease, has had a rising trend in the world and has impaired the quality of life of patients, significantly. Aeroallergens are the major cause of AR and include diverse forms of aerosols ranging from submicronic particles to relatively larger pollen grains, fungal spores, animal emanations, and biogenic debris. This research has been designed to detect the main aeroallergens responsible for AR in Tehran, Iran.

Material and Methods: In this cross-sectional study, during 2 years(2006-2007), 245 patients with AR (diagnosed based on history & physical examination), referred to Immunology-Allergy clinic of Hazrat-E-Rasul hospital , were chosen in a simple-random way and undergone skin prick test (SPT) with the common aeroallergens of the area in 2 groups of children [C: 1-12 years old] and adult [A: >12].total serum IgE levels were measured in all patients. For analysis, descriptive statistical parameters (frequency, mean…) and analytic statistical tests have been used.

Results: The mean age in adult was 29.23 years and in children, 8.76. Watery nose was the commonest complain (86.5%). Mean total serum Ig E level in adult and children was 216.75 (4-1480) and 232.71 IU/mLit (11-805), respectively. 96.3% of patients had positive SPT at least for one aeroallergen (A: 96.7%, C: 94.1%). Also, no significant statistical difference in sensitivity to aeroallergens was seen between adult and children, except for "grasses". The positive SPT in each group was as follows: House dust mites (C: 55.9% , A: 66.4%) ; Cockroach (C: 50% , A : 67.2%) ; Molds (C: 47.1% , A: 58.8%) ; Grasses (C: 64.7% , A: 82%) ; Weeds (C: 79.4% , A:88.2%) ; Trees (C: 61.8 % , A: 72.5%).

Conclusion: In this study pollen caused the most positive SPT and among pollens, Lambs Quarter (a kind of weed) was the main aeroallergen responsible for AR in our patients, with the frequency of C: 61.8% and A: 76.8%.vConsidering the pollination season of this plant in this country, advices should be made to avoid exposure to this allergen and also prophylactic medication should begin before its pollination and be continued till the end of autumn. Immunotherapy can be effective in some cases.

IMMUNOLOGY

G-protein Coupled Receptor 43 (GPR43) is Essential for Neutrophil Recruitment during Intestinal Inflammation

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Introduction: Inflammatory bowel diseases (IBD) — Crohn disease (CD) and ulcerative colitis (UC) — are disorders of unknown etiology characterized by chronic relapsing-remitting inflammation of the gastrointestinal tract. Pathophysiological and genetic evidence points to an important role of intestinal barrier function in the initiation and perpetuation of the disease. Molecular danger signals attract neutrophilic granulocytes (PMNs) to sites of infection. The G protein coupled receptor (GPR) 43 recognizes short chain fatty acids (SCFA), i.e. propionate and butyrate and is abundantly expressed on PMNs. The functional role of GPR43 activation for the orchestration of immune responses in vivo is unclear.

Material and Methods: We examined dextrane sodium sulphate (DSS)-induced acute and chronic intestinal inflammatory responses in wild-type and Gpr43-deficient mice. Clinical signs in a disease activity index, histological scoring and cytokine production in colonic tissue via ELISA-technique assessed the severity of inflammation in the colon. Chemotaxis of PMNs isolated from wildtype and Gpr43-/- mice was assessed using a transwell cell chemotactic assay (Boyden chamber).

Results: In vivo a reduced invasion of PMNs is seen in acute colitis, which is paralleled by increased mortality due to septic complications. In chronic DSS colitis, Gpr43-/- animals also show a diminished PMN migration into the intestinal mucosa but are protected from inflammatory tissue destruction. No difference in PMN migration and cytokine secretion could be detected in a sterile inflammatory model (air pouch). Ex vivo experiments show that GPR43-induced migration is dependent on the activation of the protein kinase p38α and that this signal acts in co-operation with the chemotactic cytokine KC.

Conclusion: The results indicate a critical role for GPR43-mediated recruitment of PMNs for the containment of bacterial translocation in the intestine, but also emphasize the bipotential role of PMNs mediating tissue destruction in chronic intestinal inflammation.

IMMUNOLOGY

Aberrant Splicing of Artemis in a SCID Patient can be Restored *in vitro*

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Introduction: T-ALL is a T-cell malignancy affecting children and adolescents, caused by cooperation of mutations affecting proliferation, survival, cell cycle, and differentiation.

To detect novel genomic rearrangements, we performed 1 Mb resolution array CGH with extra probes covering candidate oncogenes, as the RTKs (Receptor Tyrosine Kinase). A new fusion gene involving an RTK which functions by kinase activity and nuclear translocation, was detected in one cell line. The other gene involved is a transcription factor expressed in lymphocytes that regulates development and differentiation. Specific screening for this fusion transcript showed it's presence in 31% in a higher number of T-ALL cell lines.

Materials and Methods: constructions: we generated the sequence of the different fusion variants found in the cell lines by PCR followed by clonation into the MSCV-puromycin vector. Transfection of Hek293T cells was done with Genejuice reagent and the expression and activity level of the RTK was analyzed by Western Blotting. Screening in patients: Samples from 25 patients with T-ALL were screened for the presence of the fusion transcript at RNA level. Cell culture: T-ALL cell lines were cultured in RPMI-1640 supplemented with 20% FCS. We cultured BaF3 cells in RPMI-1640 medium supplemented with 10 % FBS and 1 ng/ml mouse IL3.By retroviral transduction and subsequent puromycin selection we made BaF3 cells express the different constructs. To test IL3-independency, stable BaF3 cell lines were washed three times in PBS and cultured in IL3-free medium. The number of viable cells was counted with a Vi-cell XR cell viability analyzer (Beckman Coulter, Fullerton, CA).
Results: The new fusion transcript was detected in 16% (4/25) of the patients.

Transduction of the constructs in BaF3 cells revealed that none had transforming capacities. Overexpression of the wild type RTK could make the cells IL3 independent after stimulation of the kinase by its ligand.

Conclusion: The presence of a fusion transcript in 31% of the cell lines and 16% of patients with T-ALL suggests a possible role in the development of leukemia.

INFECTIOUS DISEASES

Plasmid Profiling of Klebsiella Sp. and its Relation with Antibiotic Resistance at Two Hospitals of Urmia

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Introduction: Klebsiella Sp. is a group of gram negative rods and can cause different kinds of infections. Multi-drug resistant Klebsiella has been recognized as a cause of hospital acquired infections, they are resistant to numerous antibiotics, including aminoglycosides, penicillins and cephalosporins. The source of resistance in this bacterium may be chromosomal or plasmid. The aim of the present study is to compare the antibacterial susceptibility patterns with the presence of plasmids in Klebsiella isolates.

Material and Methods: 39 isolates were collected from clinical specimens submitted to two educational hospitals in Urmia/ Iran during a three months period from December 2006 until March 2007. The susceptibility patterns were determined against antibiotics, Plasmids were extracted by alkaline lysis method, electrophoresed and investigated by a UV transilluminator. Single digestion of plasmids with EcoR1 and HincII were performed and the restriction patterns were compared using a DNA ladder.

Results: The rates of resistances were determined to antibiotics as follows: gentamicin 46.1%, tobramycin 48.7%, ceftizoxime 41%, co-trimoxazole 41%, amikacin 33.3%, cephtazidime 51.3%, ciprofloxacin 30.1%, kanamycin 53.8%, nalidixic acid 30.8% and ampicillin 100%, Nitrofurantoin 41%. 25.6% of isolates harbored plasmids. Restriction enzyme analysis of plasmids showed unique pattern for all of plasmid positive isolates. There is a meaningful correlation between the presence of plasmid in isolates and resistance to the tested antibiotics.

Conclusion: The absence of plasmids from the majority of isolates showed low typeability power of this technique, so using of other molecular typing techniques in companion with plasmid profiling and restriction enzyme analysis suggested for further studies.

INFECTIOUS DISEASES

Tuberculosis and HIV Coinfections - Epidemiology, Treatment and Causes of MDR-TB in Post-Soviet Country

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Introduction: Difficulties in simultaneous treatment of HIV and TB and also inconsistent or partial therapy contribute to development of AIDS-associated multi drug resistant TB (MDR-TB). The most evident risk factor of new infections pertains to population from Post-Soviet countries. During last year in Ukraine 11.000 people died cause of TB and 680.000 still suffer epidemic. Moreover in this region almost 2% of adults live with HIV and over 60 % of HIV/AIDS deaths are attributable to tuberculosis. Situation is getting worst.

Material and Methods: Ukrainian HIV-positive and sputum smear-positive TB patients (mainly from Lvivsky Oblast) were under conventional antimicrobial treatment with rifampicin, izoniazid, pyrazinamide, ethambutol and streptomycyn. Patients were tested for HIV and MDR-TB.

Results: Due to production of pro-inflammatory cytokines by TB lessions - significant immunodepression of patients occurs. TB recurrences are more likely in patients with CD4 below 100 cell/mm3. Patients with HIV are 30 times more susceptible to developing all forms of tuberculosis.To achive the best results of treatment it is necessary to combine 5-6 drugs listed above and provide therapy during at least 15-18 months. Causes of infections: unstable condition of public health sector, insufficient finances and overpopulated prisons (for each 200.000 prisoners -12.500 have TB).

Conclusion: To avoid increasing of new MDR-TB and HIV cases in Ukraine and another Post-Soviet countries it is necessary to deliver regular and reliable treatment to TB patients, by continuation of DOTS project and urgent improvement of HIV and TB prevention.

INFECTIOUS DISEASES

Tuberculosis in Oncology Patients

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Introduction: There is a dearth of studies addressing the incidence and clinical presentation of tuberculosis (TB) in children with cancer.

Aim: To evaluate the incidence of TB in paediatric oncology patients at Tygerberg Hospital, located in a Cape Town area of high TB prevalence, and to describe the clinical characteristics of the disease in this particular group of patients whose treatment typically suppresses their immune response.

Material and Methods: We reviewed the records of 625 paediatric oncology patients admitted from 1 January 1991 to 31 December 2005. Of these, 87 received treatment for TB; however, only 57 cases had sufficient data to support a diagnosis of TB and only these were used for further analysis.

Results: In the children with TB, acute lymphoblastic leukaemia (ALL) was the most common malignancy (13/57, 22.8%). The incidence of TB in the study group was 9117/100,000/year, which is 22 times higher than the overall TB incidence reported in children from a similar background. Importantly, 47% of the active infections appeared in the 1st 5 months of chemotherapy, suggesting reactivation of latent TB.

Conclusion: Identifying latent TB in our patients and providing prophylactic treatment during the initial months of chemotherapy might have prevented disease progression in these cases. Routine screening of paediatric oncology patients for latent TB infection and exclusion of active disease prior to initiation of cancer therapy might be indicated in TB-endemic areas.

INFECTIOUS DISEASES

Prevalence of Treponematosis. Study in the Pretoria Bone Collection

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Introduction: Treponematosis is an infectious disease, which can lead to a final stage where bones can become affected. Many studies about the prevalence of bone involvement of treponemal disease are done in ancient populations, leading to the knowledge that many individuals suffered from the disease in the pre-antibiotic era. However, not much was known about the prevalence in post-antibiotic populations.

Material and Methods: With the use of 372 complete skeletons form the Pretoria Bone Collection, it was tried to obtain more information about the prevalence of treponematosis in post-antibiotic South Africa.

Results: None of the examined individuals showed specific signs of treponemal disease. Several cases with tibial periosteal bone deposition were present. For these cases the most probable diagnosis was treponematosis.

Conclusion: The fact that treponematosis still can lead to bone involvement, can have several causes. Examples of possible causes are lack in sufficient available health care, delay in ask for medical help, premature ending of the antibiotic course of infection with a resistant bacterial strain.

INFECTIOUS DISEASES

Strongyloides stercoralis Infections in Mozambique

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Introduction: Recent study shows that the prevalence of intestinal parasitic infection – including *Strongyloides stercoralis* infection – in Inhamudima, a poor suburb of Mozambique's second city Beira, is very high. However, in the local laboratory in Beira sufficient knowledge of the sensitive diagnostic methods necessary for detection of *S. stercoralis* is missing. One of the main goals of this research was to identify which diagnostic method(s) fit best in the local daily practice. The diagnostic methods compared in this study were direct smear, Ridley, Baermann and coproculture method. In recent studies real-time PCR is put forward as a – possibly very powerful – diagnostic tool in detecting *S. stercoralis*. Therefore this study also included a comparison between real-time PCR and the conventional diagnostic methods based on faecal examination. Detailed information on the prevalence of *S. stercoralis* infection – and the distribution of the infection in relation to age and gender – in Inhamudima is missing. Diarrhoea and abdominal complaints are suggested to be frequently reported symptoms in this area. Another main aim of this study was to examine the infection-index of *S. stercoralis* in Inhamudima and the distribution of the infection in relation to age and gender. One of the study's main questions also included the question as to whether – and if so, in what way – a relation can be found between *Strongyloides* infection and diarrhoea, or abdominal complaints.

Material and Methods: Eventually, 399 persons living in Inhamudima were randomly selected. Of the total study population 303 persons delivered their faeces. This accounts for a compliance of 76 %. Of the conventional methods Baermann and culture detected most *Strongyloides* infections, respectively 70 and 83 – of all 146 positives. Preferable both methods are performed in the local laboratory, for their results are complementary – one is not significantly more sensitive than the other (McNemar = 0.060). Both are significantly more sensitive than direct smear and Ridley. Real-time PCR is significantly more sensitive than *all other techniques together*. The prevalence is 5.1 times higher based on PCR, in comparison to direct smear. The prevalence is 1.3 times higher based on PCR, in comparison to all other techniques together.

Results: The prevalence of *Strongyloides* infection, within the 303 persons who delivered their faeces, based on all diagnostic methods used – including real-time PCR in Leiden – was 48 %. No significant relation was found between infection and gender, or between infection and history or presence of diarrhoea. A slight peak of infection was seen in the age group of 10-19 years.

Conclusion: Despite the promising results of PCR, it seems to have missed 12 infections, which Baermann and/or culture did detect. PCR analysis of a second Baermann and culture of all 'missed' samples were performed and these were – in all 12 cases – found negative. A possible explanation is that the 'missed' infections were very light infections – this seems to be true, for 1 - 4 L1 larvae, or maximally 80 L3 larvae were found. Another possibility is microscopic misidentification in Beira. In any case, it can be concluded that real time PCR is indeed a very powerful diagnostic tool in detecting *S. stercoralis* infection.

INFECTIOUS DISEASES

Frequency of *Heliobacter Pylori* infections Among the Patients with Ulcer and Non-ulcer Dyspepsia

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Introduction: *Helicobacter pylori* is the most important cause of duodenal and gastric ulcer. According to the results of seroepidemiological studies, more than 50 percent of the world's population is infected with this bacteria. Frequency of this infection in various areas varies depending on geographical, racial and socioeconomic conditions, and in the last 10 to 20 years, in some areas, a significant decrease in this frequency has been noted, in general population, as well as among the patients with peptic ulcer.

Aim of study is determine the frequency of *Helicobacter pylori* infection among the patients with peptic ulcer and non-ulcer dyspepsia, and to compare the results with the results of the study that was carried out 10 years ago in the same area.

Material and Methods: The research covered 163 patients (72 women and 92 men), aged 19-86 which were endoscope at The Clinic for Gastroenterology and hepatology in Novi Sad for dyspeptic disorder. All the patients underwent the routine antral biopsy and the corpus for pathological analysis, and the presence of Helicobacter pylori was determined by the coloring in the modified Giemsa method. The results were compared with those of the 1997 study, when this infection was found in 85.84 percent of patients with duodenum ulcer, 69.56 % of patients with stomac ulcer, and 72% of patients with non-ulcer dyspepsia.

Results: Out of 163 patients, 76 (46%) were detected positive with *Helicobacter pylori*. Based on endoscpic findings, three groups were formed: First group, with 75 patients with non-ulcuer dyspesion, second group of 40 patients with stomac ulcer, and third group of 48 patients with duodena ulcer. Positive findings for Helicobacter pylori wear made for 24% of patients with non-ulcer dyspepsia, 50% of patients with stomac ulcer, and 80% of patients with duodenum ulcer.

Conclusion: *Helicobacter pylori* is the most significant factor of emergence of peptic ulcer in this area.

INFECTIOUS DISEASES

Rotavirus NSP5 and NSP5-EGFP: Competition to Form Viroplasms

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Introduction: Rotaviruses (RVs) cause severe, acute gastroenteritis in infants worldwide, with a high mortality rate in developing countries. The RV genome encodes 6 structural viral proteins (VP1-VP4, VP6, VP7) and 6 non-structural proteins (NSP1-NSP6). NSP2 and NSP5 play a major role in the formation of viral factories, intracytoplasmic inclusion bodies termed 'viroplasms'. We compared the capacity of native NSP5 and of NSP5-EGFP (a fusion construct) to form viroplasms.

Material and Methods: The MA104 cell line and an MA104 cell line constitutively expressing NSP5-EGFP were used. The cell lines were infected with the bovine RV RF strain (G6P6 [1]), and infectivity was determined and calculated as tissue culture infective dose 50 % (TCID50/ml). Aliquots of infected cells were fixed at 3, 6 and 9 h p.i., reacted with primary antibodies (guinea pig) specific for NSP2 and NSP5 and the reactions visualized with Texas Red-labeled species-specific secondary antibody using confocal fluorescence microscopy. RNA extraction and agarose gel electrophoresis were used to investigate RV RNA replication.

Results: Viroplasms were visualized as red (recognizing either NSP5 or NSP2 alone), green (NSP5-EGFP autofluorescence) and yellow (superimposition of red and green). In cell cultures counterstained with NSP2, the percentage of cells with green fluorescence was highest at 3h p.i. and decreased later, red fluorescence increased over time, and yellow fluorescence increased till 6h and then decreased. In cell cultures counterstained with NSP5, the percentage of cells containing NSP-EGFP containing viroplasms (yellow + green) was also highest at 3 h. Red fluorescence was maximum at 3h, decreased until 6h and then increased. Yellow fluorescence followed red fluorescence till 3h but then stayed almost constant. The mean numbers of viroplasms/cell increased until 6 h p.i. and then remained constant.

Conclusion: The results are consistent with pre-existing excess NSP5-EGFP accumulating first in viroplasms, followed by the accumulating new wild type NSP5 outcompeting the recombinant one. Viral RNA replication and infectivity yield of progeny virus were reduced in cells containing NSP5-EGFP, implying that it was less efficient at contributing to viral production than the native protein. NSP5 oligomerises and also interacts with NSP2 through its C terminus; the attachment of the EGFP at the C terminus may inhibit these functions. The work suggests that NSP5-EGFP is less efficient in promoting virus production and that homo-oligomerisation of native NSP5 and its interaction with NSP2 are important for its function.

INFECTIOUS DISEASES

Reasons for and Outcomes of Hospital Admissions for HIV-infected Children at the Korle Bu Teaching Hospital, Accra, Ghana

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Introduction: The burden of pediatric HIV infection remains high in resource-poor settings. A high burden of opportunistic infections could lead to high mortality as healthcare delivery systems are already overwhelmed. The primary objective of this study was to determine the reasons for hospital admissions of HIV-infected pediatric patients to a tertiary teaching hospital and the outcome of these hospital admissions over a 1-year period.

Material and Methods: Retrospective chart review of all HIV-infected children aged 0 to 13 years of age admitted to the pediatric unit at Korle-Bu Teaching Hospital from 30 June 2007 to 30 June 2008. A chart abstraction form was used to gather data on age, gender, weight, presenting conditions, diagnosis, duration of hospital stay, ARV treatment, outcome, and other critical clinical information.

Results: A total of 102 admission occurred among 76 children. The mean age of the children was 4.5 (± 3.79) years and 55% were males. At the time of admission, 55 patients (64%) had a weight for age < 2nd percentile and 29 patients (28%) had a weight for age between the 2nd and 25th percentile. A majority of children (67%) were not on antiretroviral therapy at time of admission. Twenty three (30%) of the 76 children were diagnosed with HIV infection during a hospital admission. Of the 102 admissions, the predominant diagnosis included pneumonia (44%), gastroenteritis (24%), pulmonary tuberculosis (22%), and/or malaria (19%). The most common presenting conditions on admission included fever (68%), cough (54%), vomiting (50%), and/or diarrhea (34%). Death occurred in 12 (12%) of the 102 admissions. Pneumonia, gastroenteritis, malnutrition and septicemia were the common causes of death. Age, gender, and admitting diagnosis were not associated with death or duration of hospitalization > 7 days.

Conclusion: Failure to thrive and common endemic infections but not opportunistic conditions were the predominant reasons for hospitalization and death for pediatric HIV/AIDS patients in this tertiary hospital. Clinicians caring for HIV-infected children should be cognizant of these infections and institute appropriate early treatment or provide preventative measures. Hospitalized children with these conditions should be tested for HIV infection if their status is unknown. We did not find any significant predictors of in-hospital mortality in this small cohort.

INFECTIOUS DISEASES

Potential Role of CD133/CD117 Positive Stem Cells in Liver fibrosis Caused by *Schistosoma mansoni* Infection

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Introduction: *Schistosoma mansoni* infections in rodents generate liver pathology in the form of fibrosis – that is dependent on the genetic background of the mice strain. Chronic inflammation is associated with angiogenesis, whereby circulating endothelial progenitor cells play an important role. Such cells originating from the bone marrow can be distinguished into two major groups based on the stem cell markers CD133/prominin and CD117/c-kit. Partial hepatectomy leads to the mobilization of a unique population of progenitor cells with the potential to differentiate into hepatocytes in vitro and a probable role in liver regeneration. An important part of such stem cells are CD133 positive. Stem cell factor (SCF) and its receptor c-kit also play an important role in liver regeneration after almost 70% hepatectomy. There is however little known about the roles of CD133 and CD117 positive stem cells during S. mansoni induced liver fibrosis.

Material and Methods: Using immunohistochemical techniques the expression of these stem cell markers and CD34 (endothelial marker detecting newly formed blood vessels) were studied. Frozen sections from spleen, liver, kidney and brains of infected mice strains were screened at acute (8 weeks) and chronic (16 weeks) stages of pathology.

Results: Varying levels of expression were noted in kidneys and in the liver. The inbred mice strain C57BL6 depicted light pathology upon S. mansoni infection due to a balanced Th1/Th2 immune reaction to the parasite; in contrast the infected C3H mice developed serious pathology due to strong Th2 responses. In such an environment administration of CD133+/117+ cells in vivo could result in the creation of an immunologically balanced environment in the liver with diminished fibrosis and resulting liver regeneration. Further research is ongoing.

Conclusion: In normal situations, the host immune system is largely incapable of resisting primary infection with the Schistosoma parasite, and resistance to superinfection takes years to develop. So, the survival of the host seems to depend on the ability to make an appropriately balanced T-helper response that is able to orchestrate granuloma development, and minimize fibrosis and severe morbidity during chronic infection. In this context, the CD133+/CD117+ stem cells provide a novel therapeutic possibility.

INFECTIOUS DISEASES

Prevalence of Hepatitis Delta Virus Infection in Various Groups of HBV Infection in Imam-Khomeyni Hospital, Tehran (2006-2007)

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Introduction: Hepatitis B virus infection is an important cause of liver morbidity and mortality worldwide. HDV changes the natural course of HBV. The prevalence of HDV infection was not determined in the various groups of HBV infection [carriers, acute hepatitis, chronic hepatitis, cirrhosis, and hepatocellular carcinoma(HCC)] in Iran.

We aimed to research the prevalence of hepatitis D virus infection in various groups of HBV infection in Imam-Khomeyni hospital of Tehran (2006-2007).

Material and Methods: Serological markers of HBV and HDV infection [HBs Ag, HBe Ag, anti HBe Ab, anti HDV Ab(Ig M, Ig G)] were determined by ELISA test in 206 patient with HBV infection who referred to Imam-Khomeyni hospital. These patients categorized to asymptomatic carriers, acute hepatitis, chronic hepatitis, cirrhosis, and HCC according to history, physical examination, and lab findings.

Results: HDV infection was detected in 12.6%(26/206) of HBV infected patients. It was detected in 1.6%(1/62) of asymptomatic carriers, 20%(1/5) of acute hepatitis, 5.6%(5/88) of chronic hepatitis, 37.2%(16/43) of cirrhosis, and 37.5%(3/8) of HCC patients. HDV infection showed a five fold increment in chronic hepatitis ($p<0.05$) and ~16 fold increment in cirrhosis ($p<0.001$) compared to HDV infection in asymptomatic carriers. HDV infection was equally distributed between sexes. Mean ages of HDV carriers, acute hepatitis, chronic hepatitis, cirrhosis, and HCC were (28), (33), (39.5±21), (47.1±11.5), (58.6±9.2) year respectively.

Conclusion: The prevalence of HDV infection were 12.6%, the higher prevalence of HDV infection in more severe forms of hepatitis B virus infection suggests that HDV infection increases the severity of chronic hepatitis B. HDV infection remains a major cause of chronic liver disease in Tehran in spite of its decreasing prevalence in countries such as Italy.

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INFECTIOUS DISEASES

Antitrichomonal Activity of Plant Extracts from Family Lamiaceae (*Coleus blumei, Origanum vulgare*, & *Vitex negundo*): *In Vitro* Study

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Introduction: Trichomoniasis is an infection caused by the protozoan *Trichomonas vaginalis*, and is ranked as the most common nonviral sexually transmitted disease (STD) in the world. A recent Philippine study on the prevalence of trichomoniasis estimated an infection rate of 37% among Filipino women. Efforts to contain and alleviate this problem remain a continuing initiative of government health institutions. The Philippines' numerous ecological niches provide for a vast backdrop from which effective and accessible plant herbal remedies can be obtained. One promising source of antitrichomonal extracts is the family Lamiaceae, whose members have already been shown to exhibit antibacterial, antiviral, and antiprotozoal activities. In the search for an alternative to conventional therapeutic regimen, the study was conducted to determine the activities of three crude ethanolic plant extracts from family Lamiaceae (i.e., *Coleus blumei, Origanum vulgare,* and *Vitex negundo*) against *T. vaginalis*. The study also aimed to characterize the biochemical components of the most potent plant extract, and determine its toxicity in mice.

Material and Methods: Crude ethanolic extracts of the plants were obtained and a minimum inhibitory concentration (MIC) assay was done using varying concentrations of *C. blumei, O. vulgare* and *V. negundo*. Phytochemical screening of *C. blumei* was performed by the College of Pharmacy, University of the Philippines – Manila. A mice toxicity assay of *C. blumei* was also done by intraperitoneal injection of the extract. Measures were taken to ensure that the least amount of pain was inflicted on the animals, and proper decapitation and disposal procedures were observed.

Results: The *C. blumei* crude extract exhibited the highest activity among the treatments, with a minimum inhibitory concentration (MIC) of 2 mg/mL, an activity not significantly different from metronidazole ($p = 0.809$), the drug of choice for trichomoniasis. Phytochemical analysis of the *C. blumei* extract indicated the presence of glycosides, plant acids, reducing agents, and alkaloids. Some of these substances have been reported to possess antitrichomonal or antiprotozoal activity, and would likely account for the observed activity of the *C. blumei* extract. The mice toxicity assay revealed minimal side effects even at several magnitudes of concentration (250-500X) higher than the MIC, suggesting a wide margin of safety for the crude extract.

Conclusion: These initial findings warrant further studies to isolate, purify, and elucidate the active antitrichomonal product present in the *C. blumei* extract, and to explore its potential as an alternative STD medication for human use.

INFECTIOUS DISEASES

Side Effects of HAART in HIV-1 infected Pregnant and Non-pregnant Women

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Introduction: We evaluated the risk of hepatotoxicity and rash in treatment naïve HIV-1 positive pregnant and non-pregnant women, who started HAART in the Netherlands between 1997 and 2008.

Material and Methods: Data were used from the ATHENA observational cohort. The study population consisted of HIV1-positive naïve pregnant and non-pregnant women between January 1997 and February 2008. Demographic, treatment and pregnancy related data were collected. Risk of side effects during pregnancy was determined using univariate and multivariate logistic regression. Multivariate models were built using forward-stepwise techniques. A p-value <0.05 was considered statistically significant. Analyses were adjusted for age, region of origin, baseline HIV-RNA and CD4 cell counts, HAART regimen and hepatits B and C co-infection.

Results: 485 Pregnant and 1156 non-pregnant women were included. Pregnant women were younger at the time of HIV diagnosis (28 versus 33 years), more often originated from Sub Sahara Africa (SSA) and were treated more often with a PI based regimen (70% versus 42%). Controls were more often treated with a NNRTI based regimen (51% versus 21%). The adjusted risk of hepatotoxicity was higher among pregnant women compared to controls (OR 4.31, CI 95% 1.29-14.40, p=0.02). The risk of skin rash was similar in pregnant and non-pregnant women. Sub-Saharan Africa origin was independently associated with a significant lower risk of rash compared to West-European origin (OR 0.31, CI 95% 0.15-0.63, p=<0.01.

Conclusion: Pregnancy is associated with a higher risk of hepatotoxicity. The risk of rash was associated with region of origin, and not with pregnancy.

INFECTIOUS DISEASES

Caesarean Sections Rate, Indications and Outcome among Women admitted at Muhimbili National Hospital From July 2007 to June 2008

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Introduction: Based on historical data, a Caesarean Delivery (CD) rate of 5 to 15 percent appears to give the best maternal outcomes; the rate for the best infant outcomes is less clear but is likely far higher. The CD rate worldwide is 15 percent of births, 21.1 percent rate in developed countries, but is only 2 percent is the least developed countries. The steadily increasing global rate of caesarean section has become one of the most debated topics in maternal care.

Objective: To determine caesarean section rate, indications and outcome among women who had child delivery at M.N.H. from July 2007 to June 2008.

Materials & Methods: A retrospective descriptive study. The study was conducted at M.N.H. from July 2007 to June 2008. The study population included all women who delivered during the study period, and the study sample included women admitted to M. N. H post – caesarean section. Data was obtained from the Patients' medical records and analyzed by Epi Info version 3.5

Results: A total of 9,211 child births included; caesarean delivery rate was 42.2 percent of which 95.1 percent were singleton. The mean age was (28.46+9.26) years, 39.8 percent of the mothers were primiparae and 4.5 percent grand multiparae. All had registered for antenatal care in any health institution. The leading indications for caesarean delivery were: prolonged/obstructed labor (46.1%), previous caesarean section (26.5%) and fetal distress (11.9%) with a (p value < 0.05). Maternal deaths rate was (0.4%), the main causes being eclampsia (57.1%) and post partum hemorrhage (28.6%). There were also 138 (3.6%) neonatal deaths, 62 (1.6%) macerated stillbirths, and 110 (2.8%) fresh stillbirths.

Conclusion: The rise in caesarean delivery rates at M.N.H. is relatively high (from 15.8% in 1999, 31.8% in 2004 to 42.2% in 2007/2008). However, there are no changes in the main indications for caesarean section and the risk of caesarean delivery remains to increase among primiparae mothers.

There is a need for studies to be done to determine the causes for this steadily rising caesarean section rate. Furthermore, this may necessitate the call for a careful cost – benefit analysis to be done to see if any meaningful interventions can be implemented.

INTERNAL MEDICINE

Assessment of Insulin Resistance and Effect of Metformin in Non Alcoholic in Steatohepatosis

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Introduction: Insulin resistance plays a major role in the pathogenesis of non alcoholic steatohepatitis (NASH). Insulin-sensitizing drugs like metformin may have a role in treatment in this disease.

Objective: To determine insulin resistance and role of metformin in the treatment of NASH.
Material and Methods: We prospectively studied 25 patients with NASH over a period of one and half years. In addition to clinical pathological profile, we studied the insulin resistance by insulin tolerance test in 10 of them; seven of them, who did not respond to 3 months of low-calorie, low-fat diet, exercise, weight reduction with metformin for six month. Results were compared with control groups.

Results: All 10 patients with NASH tested had low insulin sensitivity; there was significant difference in the rate constant for insulin sensitivity (kit) between patients with NASH and normal volunteers. Thirteen (52%) patients responded to dietary restriction, exercise, weight reduction and UDCA. Four of 7 patients treated with metformin had normalization of ALT.

Conclusion: Patients with NASH have insulin resistance. Metformin may have a role in treatment of these patients.

INTERNAL MEDICINE

Association of Polymorphisms in Interleukin-4 and Interleukin-10 Genes with Graves' Disease in Iranian Patients

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Introduction: Graves' disease (GD) is the most common form of autoimmune thyroid disease with a still largely unclear etiology. Among genetic factors that may contribute to the development and progression of the disease and its complications are polymorphisms in the genes encoding cytokines. In the present study, we focused on gene polymorphisms of two important cytokines, namely interleukin-4 (IL-4) and IL-10.

Material and Methods: Venous sample of participants were obtained and DNA was extracted by salting out method. Using PCR-restriction fragment length polymorphism method (PCR-RFLP), the association between GD and the following polymorphisms was studied in 107 patients and 140 healthy controls: IL-4 (-1098T/G, -590T/C, -33C/T), and IL-10 (-1082A/G, -819C/T, -592A/C).

Results: For IL-4, the -1098G allele, TG genotype and GG genotype increased the odds of having the disease 3.08- ($P < 0.0001$), 2.56- ($P = 0.0041$) and 105-fold ($P < 0.0001$), respectively. The -590T allele and TC genotype decreased the odds of having the disease 2.13- ($P < 0.0001$) and 12.5-fold ($P < 0.0001$), respectively. The -33T allele and TT genotype increased the odds of having the disease 2.52-fold ($P < 0.0001$) and 118.83 ($P < 0.0001$), respectively. For IL-10, the -1082G allele and GG genotype increased the odds of having the disease 2.16- ($P < 0.0001$) and 2.05-fold ($P = 0.0246$), respectively. The -819T allele, TC genotype and TT genotype increased the odds of having the disease 2.16- ($P < 0.0001$), 2.09- ($P = 0.0114$) and 4.58-fold ($P = 0.0003$), respectively. The -592C allele and CC genotype decreased the odds of having the disease 2.44- ($P < 0.0001$) and 5.56-fold ($P < 0.0001$), respectively.

Conclusion: This study showed the significant association of polymorphisms in IL4 and IL10 with graves disease. Future studies are needed to confirm our findings.

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INTERNAL MEDICINE

The Validation of IGF-I and P-III-P Assays in the Development of a Methodology to Detect Growth Hormone Abuse in Sport

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Introduction: It is believed Growth hormone (GH) and Insulin-like growth factor-I (IGF-I) are abused by athletes for their anabolic and lipolytic properties. There is currently no test to detect doping with IGF-I and the test for GH abuse can only detect individuals who have taken GH in the last 36 hours.

The GH-2000 team developed formulae based on serum IGF-I and P-III-P levels that can determine if an individual is taking GH in up to 90% of cases. These formulae were developed using the Nichols IGF-I assay and the CIS P-III-P immunoassay, unfortunately the Nichols assay is no longer available. The relationship of other IGF-I assays to each other and to Nichols is vital in being able to apply these formulae to values achieved from the currently available IGF-I assays. The CIS P-III-P assay measures in different units to the other commercially available P-III-P assay Orion, this in itself is not a problem and is akin to measuring weight in stones or kilograms but it is important to know the relationship between them.

Material and Methods: One hundred and twenty four serum samples were assayed for IGF-I with two commercially available immunoassay kits (DSL and Immunotech) and for P-III-P with two further radioimmunoassays (CIS and Orion). The body composition of eleven amateur athletes was estimated using air displacement plesthymography (BODPOD), bioimpedance and skinfold thickness. Physical fitness was estimated by a maximal treadmill test following the Bruce Protocol.

Results: Serum IGF-I to P-III-P showed a positive correlation of $r = 0.388$ and $p \leq 0.01$.Serum IGF-I to body fat percentage by all estimates showed no significant relationship with all $r<0.1$ and all p values over 0.7. I found the DSL to immunotech conversion factor to be Immunotech = 0.7828 X [DSL] and CIS to Orion conversion to be Orion = 9.2057 X [CIS] giving similar results to previous work done in this area. This work allows values achieved from current IGF-I and P-III-P assays to be converted into values that can be used in the GH-2000 formulae to detect GH abuse.

Conclusion: The development of conversion factors for the commercially available immunoassays takes us one step closer to a reliable test for rhGH abuse.

INTERNAL MEDICINE

The Correlation Between LH to FSH Ratio and Metabolic Factors in Indonesian PCOS Patients; Preliminary Study

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Introduction: Polycystic ovarian syndrome (PCOS) is common endocrine disorder affecting female fertility, including in Indonesia. Metabolic factors are related with the clinical manifestation of PCOS (i.e. insulin, glucose levels, Body Mass Index). From the previous study, LH to FSH ratio has been used as a value that correlate with degree of severity in patient with PCOS. Other factor that may contribute to clinical sign is area under curve of Insulin (AUC-I). This research is proposed to see which factor mentioned above that might has stronger correlation with LH to FSH ratio in PCOS patient in Indonesia.

Material and Methods: The data were collected in Cipto Mangunkusumo General Hospital and Hermina Maternal and Child Hospital from January till November 2008. These patients were physically examined and followed by serum LH, FSH, Fasting glucose, fasting insulin and 2 hours postprandial insulin measurement. AUC-I was calculated by using positive incremental formula.

Results: The strong correlation between LH to FSH ratio and fasting insulin was observed in this study (r: 0.663, $P<0.05$). In addition, we also observed strong correlation between LH to FSH ratio and AUC-I (r: 0.639, $P<0.05$). During this study we did not find any correlation between LH to FSH ratio and fasting glucose level (r: 0.342, $P>0.05$); LH to FSH ratio and BMI (r: 0.323, $P>0.05$); and LH to FSH ratio and 2 hours postprandial insulin (r: 0.228, $P>0.05$).

Conclusion: The result of our study shows that the increasing of LH to FSH ratio shows a strong correlation with the fasting insulin and AUC-I. The increasing of insulin has an independent correlation with glucose level, BMI and 2 hours postprandial insulin. This result is similar with the previous study that shows increasing insulin had a stronger correlation with abdominal obesity than BMI. Glucose levels have no correlation with the increasing LH to FSH ratio because most of the PCOS patients have a normal glucose levels. The increasing of LH to FSH ratio has a strong correlation with AUC-I but not with 2 hours postprandial insulin. It is suggested the result measurement of 2 hours postprandial insulin shows a wide variation. The conclusion of our study is that fasting insulin and AUC-I has a strong correlation with increasing LH to FSH ratio, independent to glucose levels, BMI and 2 hours postprandial insulin.

MOLECULAR AND CELL BIOLOGY

RNA Interference and MicroRNAs: New Regulation of Proteome and its Analysis in B-Chronic Lymphocytic Leukemia

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Introduction: MicroRNAs (miRNAs) are small RNA molecules acting as post-transcriptional regulators of gene expression. They are dynamically regulated during cell differentiation, proliferation and apoptosis. Many studies have shown that miRNAs are aberrantly expressed in cancer cells, suggesting that they might act as a novel class of oncogenes or tumor suppressors (reviewed in Kusenda, Mráz et al., 2006). Although discovery and functional characterization of miR15-miR-16 at 13q14.3 clearly demonstrated involvement of microRNAs in indolent CLL pathogenesis (del of 13q), the functional role of microRNAs in aggressive CLL was not clearly defined. The aim of this study was to identify miRNAs, which could have a potential role in B-chronic lymphocytic leukemia (CLL) pathogenesis and might be useful as prognostic markers.

Material and Methods: Peripheral blood samples from 30 B-CLL patients were collected (del/mut p53 n=13: unmut IgVH n=8; mut IgVH n=3, polyclonal n=2; wt p53 n=17: mut IgVH n=9; unmut IgVH n=8). B-CLL lymphocytes were separated by RosetteSep (obtained purity was >95% of CD5+19+ B-CLL cells). Real-time PCR (ABI TaqMan MicroRNA Assays) was used to detect the expression of 35 microRNAs, which were chosen based on target prediction and literature data. The obtained values were correlated with functional status of the p53 (deletion/mutation of p53 or wt p53) and other known standard prognostic markers.

Results: Author has primarily focused on the identification of microRNAs abnormally expressed in a CLL subtype with poor prognosis harbouring a deletion/mutation of the p53 gene. I have observed a statistically significant down-regulation of miR-34 in the p53-abnormal samples (p=0,0001). MiR-34 has been recently reported to be directly regulated by p53 protein in vitro and these data show, to our knowledge for the first time, that this microRNA is abnormally expressed in leukemic patients and its expression is directly dependent on the p53 functional status. In addition, several other microRNAs were identified (miR-29 family, miR-23 family, miR-17-5p, miR-15a, miR-221), whose expression correlates with CLL prognosis and abnormal p53 function.

Conclusion: These results show that microRNAs expression varies in the CLL prognostic subtypes and discussed miRNAs could be potentialy used as markers of disease prognossis. CLL patients with a deletion/mutation of the p53 gene display significantly lower expression of miR-34 targeting anti-apoptotic gene Bcl-2 (this protein is known to be associated with CLL pathogenesis). Consequently, this miRNA could potentially play an important role in the pathogenesis of chronic lymphocytic leukemia subgroups.

Mraz et al. 2009, Leukemia; Mraz et al. Leukemia and Lymphoma, in press
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MOLECULAR AND CELL BIOLOGY

Senescence Prone Phenotype after Cellular Stress in Fibroblasts Obtained from Elderly

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Introduction: Apoptosis and cellular senescence are the cellular responses to a variety of stresses. Both, apoptosis and senescence have been proposed to contribute to the ageing phenotype. To maintain tissue homeostasis, cells lost by apoptosis will be replaced by other cells, whereas senescent cells stay metabolically active in tissue compartments. When the self renewing capacity of a tissue is diminished, it can be argued that senescence would be a preferable 'cell saving' mechanism. Until now, the in vitro relation between apoptosis, cellular senescence and donor-age remains uncertain, therefore we investigated cellular responses to rotenone in young healthy individuals and nonagenarians.

Material and Methods: Fibroblast cultures were established from skin biopsies obtained from participants of the Leiden 85+ Study (n=10) and subjects, aged 20 years (n=10). Markers for senescence and apoptosis were measured in non-stressed conditions and after treatment with 600nM rotenone for three days. Cellular senescence was assessed by using a novel method measuring Senescence Associated β-galactosidase (SA β gal) activity by flow cytometry with C12FDG as a fluorescent substrate of SA β gal. SA β gal activity was expressed as the median fluorescence intensity (MFI). Apoptosis was assessed by Propidium Iodide and Annexine V flow cytometric measurements. Early apoptotic cells were defined as Annexine V positive, PI negative cells expressed as percentage.

Results: SA β gal activity in non-stressed conditions was significantly higher in strains obtained from nonagenarian donors compared to younger subjects (MFI (SD) 2869 (982) vs 2007 (666), p=0.036). After rotenone treatment, this difference increased (MFI (SD) 4317(953) vs 3199(827), p=0.012).
The percentage of apoptotic cells in non stressed conditions were not different between young and old donors (% (SD) 0.89 (0.42) vs 0.74 (0.34) p=0.389). After treatment with rotenone, fibroblast strains obtained from nonagenarian donors had a significantly lower percentage of apoptotic cells compared to the younger subjects (% (SD) 4.89 (0.997) vs 6.23 (1.33) p=0.021).

Conclusion: The increase in SA β gal activity with age in non stressed conditions might reflect the increase in the amount of senescent cells with age in vivo. This is consistent with the previous described findings in human and baboon skin. After treatment with rotenone, fibroblasts from young subjects undergo apoptosis more readily than do cell strains obtained from older donors, indicating preference for senescence pathways to apoptosis with chronological age.

MOLECULAR AND CELL BIOLOGY

Changes in the Antioxidant Defence System and Nitric Oxide Signal System on the Experimental Model of Acute Pain Syndrome Development

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Introduction: The main cause of pain syndrome development is the complex of complicated pathophisiological mechanisms. Changes in the antioxidant defence system and nitric oxide signal system (NO) are very closely combined. Nowadays, the investigation of pain syndrome takes an important role and it doesn't have the adequate therapy.

Material and Methods: Ten Wistar male rats weighting 180-200 grams have been investigated. The pain syndrome was modeled by cutting ischiadic nerve under the ethereal anesthesia. The samples of peripheral blood, brain and spinal cord were taken on 72 hours after animals were euthanased. The state of NO according to the Green L.C.,David A.W.(1982);and the state of Antioxidant system was evaluated on the basis of reduced glutathione concentrations, which we estimated on the basis of the reaction with Elman reagent (1959); activity of catalase according to Wheeler et al. (1990).

Results: We revealed the increase of NO stable metabolites production to 1.11± 0.07 μmole/g of protein (control 0.93±0.04 μmole/g of protein) in the brain; to 8.25±0.3 μmole/g of protein (control 3.86±0.11 μmole/g of protein) in the spinal cord; to 13.36±1.04 μmole/mg of hemoglobin (control 15.36±1.17 μmole/mg of hemoglobin) in the hemolisate. The activity of catalase in the brain and spinal cord increased in the comparison with control from 2.03×10-3±0.01 μmole H2O2×min/mg to 4.02×10-3±0.01 μmole H2O2×min/mg in the spinal cord; from 0.03±0.001 μmole H2O2×min/mg to 0.04±0.002 μmole H2O2×min/mg in the brain; from 8.49±0.75 μmole/mg of hemoglobin to 12.04±0.49 μmole/mg of hemoglobin in the hemolisate. The level of reduced glutathione in homogenate of brain and spinal cord of experimental animals also increased and was 0.75±0.001 μmole H2O2×min/mg (control 0.32±0.002 μmole H2O2×min/mg) in the spinal cord; 0.21±0.03 μmole/g (control 0.13±0.01 μmole/g) in the brain correspondingly.

Conclusion: The system of NO plays a very important role in the reaction of adaptation. As a result of adaptation reactions development both at local and systemic levels concentration of reduced glutathione and activity of catalase increased. The changes of given parameters are increased in the brain and spinal cord tissues. This fact proves the presence of integral response of all central nervous system both segmental and suprasegmental levels while forming compensatory response on intensive pain irritation.

NEPHROLOGY AND UROLOGY

Comparison of Glomerular Filtration Rate Measurements Using ^{125}I-Iothalamate and ^{131}I-Hippuran

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Introduction: Treatment and prognosis of chronic kidney disease (CKD) are adjusted to the glomerular filtration rates (GFR) of the patient, so an accurate method to estimate the GFR is most important. Without the use of a bladder catheter incomplete urine collection take place and the GFR will be underestimated when using the clearance of exogenous markers, like ^{125}I-iothalamate. With the use of ^{131}I-hippuran there can be corrected for this incomplete urine collection. The aim of this study was to evaluate if we need to correct for incomplete urine collection with ^{131}I-hippuran. Furthermore, subgroup analyses for body weight, age, gender and GFR were performed.

Material and Methods: 332 Adult patients who underwent a GFR measurement between January 2003 and December 2006 at the Academic Medical Center in Amsterdam were included. Only the last GFR measurement available was included and the GFR had to ≥15 mL/min per 1.73m^2. GFR was measured with ^{125}I-iothalamate by the standard method with correction for incomplete urine collections using ^{131}I-hippuran. Bland-Altman analyses as well as regression analyses were performed and the intraclass correlation coefficient (ICC) was calculated to asses the repeatability within and between the different GFR measurement methods.

Results: We founded a stronger association between the corrected GFR measurements than between the uncorrected GFR measurements. The ICC was 0.8 versus 0.98 (uncorrected versus corrected GFR method). Accuracy and precision were better within the corrected compared to the uncorrected GFR method. The association between the different GFR methods was good, Cohen's kappa was 0.81 and the ICC was 0.95. Accuracy and precision between the different GFR methods were 3.22 mL/min per 1.73m^2 ± 10.94 mL/min per 1.73m^2. The accuracy and precision became worse with rising GFR within the uncorrected GFR method, within the corrected GFR method and between these two GFR methods. The accuracy en precision became better with rising age within the uncorrected GFR method. The accuracy and precision became worse with rising weight in women within the uncorrected GFR method and between these two GFR methods.

Conclusion: The corrected GFR method was more precise and accurate then the uncorrected GFR method. The agreement between the two different GFR methods was good but the precision was poor. Therefore we recommend correcting for incomplete urine collection with ^{131}I-hippuran especially in patients which suffer from micturate problems.

NEPHROLOGY AND UROLOGY

Balkan Endemic Nephropathy in Bosnia and Herzegovina

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Introduction*:* Balkan Endemic Nephropathy (BEN) is a chronic, irreversible renal disease of unknown origin, geographically confined to several rural regions of the Balkan Peninsula. Because it leads to inevitable terminal renal insufficiency requiring renal replacement therapy (RRT), it represents a serious problem for public health.

The aim of this paper is to present and analyse epidemiological facts about patients with BEN diagnosis on chronic dialysis treatment in Bosnia and Herzegovina.

Material and Methods: In this study we used data from The Renal Registry of Society of Nephrology, Dialysis and Transplantation of Bosnia and Herzegovina for 2003-2006 period. Basic information collected from patients were: sex, age, place of birth and address, primary renal disease, date of the first dialysis treatment, history of urothelial tumor and family history of similar kidney disease and RRT. Statistical analysis: prevalence and incidence of the patients.

Results*:* In 2006, 23 dialysis centres (DC) reported totally 2196 patients on chronic dialysis treatment (duration: more than 90 days). There were 328 (14,94%) patients with BEN diagnosis in Bosnia and Herzegovina, 310 of them (94,51%) in four DCs in North-Eastern Bosnia: Brcko, Samac, Bijeljina and Odzak. We found almost the same number of male and female BEN patients. The youngest reported patients were two women of the age of 43, and the oldest patient is 87. In 2006, 72,86% of patients were older than 65. Urothelial tumor was reported in 42 from 328 BEN patients (12,80%). 149 BEN patients (45,43%) confirmed similar kidney disease in their family, but 79 (24,08%) have had a family member on RRT. There was a very small number of BEN patients in Bosnia and Herzegovina with a kidney graft, namely 1 patient in 2006. Mortality of the BEN patients was 13,11%.

Conclusion*:* Based on the presented facts it is evident that BEN prevalence in Bosnia and Herzegovina in the 2003-2006 period shows slow increase, but it is stable. Ben is still a big medical and social problem in Bosnia and Herzegovina, especially in the North-Eastern Bosnia. Therefore it is necessary to make a detailed study of BEN and take adequate health care of these patients.

NEUROLOGY

Relationship Between Tumour Grade and Fatigue in Glioma Patients

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Introduction: Fatigue is an important problem for glioma patients. The exact mechanism of fatigue in cancer is completely unknown, but both the tumour itself and its treatment may contribute to fatigue. It is unknown whether tumour grade influences fatigue and therefore it is not yet possible to predict levels of fatigue by tumour grade.

Material and Methods: This study was conducted at the Western General Hospital in Edinburgh. Subjects were identified by their patient records and invited to participate if they had supratentorial glioma which was histologically verified after primary surgery. Patients who consented to participate were administered a visual analogue scale to assess the influence of fatigue on daily life.

Results: 21 of 51 participants (41.2%) reported that fatigue had major impact on their daily life. The odds ratio for fatigue for high grade glioma compared with low grade glioma was 0.889 (p=0.838), meaning that patients with a high grade glioma have a smaller risk of fatigue influencing daily life. After adjustment for 'radiotherapy in the last six months' the odds ratio for fatigue was 0.857 (p=0.802).

Conclusion: The influence of fatigue on daily life is not significantly different between patients with low grade gliomas and patients with high grade gliomas, even after adjustment for the influence of radiotherapy in the last six months.

NEUROLOGY

Perceptual Closure and Thalamic Networks in Schizophrenia

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Introduction: Individuals are able to recognize common objects even when portions of them are obscured from view, reflecting the operation of neural perceptual closure processes. Perceptual closure is impaired in patients with schizophrenia, leading to deficits in object recognition. We suppose that impaired perceptual closure could start from the lack of integrity of basic sensory processing in schizophrenia and further more, this abnormality could start at the thalamic level. Our aim is to theoretically test, using computer simulated thalamic neuronal networks, if this failure in reconstructing images is due to visual stimuli integration at thalamic level.

Material and Methods: 8 patients with schizophrenia were recruited. Written informed consent was obtained. Pictures used in our study were manipulated to produce six incrementally fragmented images "Stage 1" refers to a complete picture (ink line drawing), and "stage 6" refers to the most fragmented version of an object. Pictures were seen by the patients and by the control group for the first time. The impairment in object recognition, in schizophrenic patients and their need for more complete pictures was evaluated in comparison with the control group. In parallel, the thalamic network was reconstructed in respect with the in vivo and in vitro existing data. Then, on the reconstructed thalamic network (using Neuron, open source) we operate changes in respect to the findings published on the thalamic function and morphology in schizophrenic patients. We assume that "stage 6" is transmitting less total information to the thalamus but using more spike trains because of the "novelty" of the context and because there is not any priming effect or word prompting. For the "stage 1" we assume the opposite input pattern to the network.

Results: The thalamic cells respond in a different manner depending on the input. Also, together with the thalamic reticular cells, the network response depends on the firing rates and also depends on the nature of the previous spike train. Then we analyzed this result in respect with the need of the schizophrenic patients to request a more complete image for naming the object and in respect with the encoding pattern used in the network.

Conclusion: Although the cognitive processes are hard to replicate, our model of correspondence used in this study could be a starting point in the process of elucidating the basic sensory processing in schizophrenia.

NEUROLOGY

Microvascular Anatomy of Trigeminal Nerve in Correlation with Age

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Introduction: Trigeminal Neuralgia (TN) is a neurological disorder that clinically presents as facial pain syndrome. Etiology of TN has not been definitely explained; the most popular is neurovascular compression theory. According to this, the compression of Trigeminal Nerve Root results in pathological changes in its structure,therefore producing neuralgic pain. TN usually affects people over 50 years. Contact between nerve and vessel is commonly found in people. The aim of our study was to characterize microvascular relationships of Trigeminal Nerve and correlate it with age of patients.

Material and Methods: Microvascular relationships were examined on 30 brain specimens obtained during routine autopsies from both men and women at the age of 22 to 98. After injecting arteries and veins with coloured medium, specimens were fixed in 7% formaldehyde solution.

Microsurgical technique was used and measurements were taken with the ocular ruler of the operating microscope. Second part of the study was done on MRI images of 30 brains of randomly chosen adults not suffering from TN at the age of 20 to 80. Anatomical relations of Trigeminal Nerve Root were evaluated.

Results: Our study showed increasing tortuosity and elongation of Superior Cerebellar Artery (SCA) and Anterior Inferior Cerebellar Artery (AICA) with age what resulted in shortening the distance between the mentioned arteries and Trigeminal Nerve. In 27 cases (47%) we observed an artery in contact with the nerve. 70% of these occured in specimens from people over 50; the most commonly SCA was the offending vessel. SCA was in contact with superior and medial aspect of the root. The inferior and lateral aspect was most often affected by AICA. Considering relationship with veins we found no correlation with age. Contact between veins and nerve was found in 10 cases (17%) and was formed by transverse or lateral pontine veins affecting the lateral, inferior or medial side of the nerve. In radiological part of the research, neurovascular contact occured in 42%.

Conclusion: The study showed that microvascular relationships of Trigeminal Nerve were age-dependent and may predispose older patients to neurovascular conflict. Our study also confirms that in pain-free population neurovascular contact is quite common. According to our observations and some authors, not neurovascular contact itself, but rather consequences of it, like deterioration of the nerve fibers, could be the cause of neuralgia, what could explain presence of the contact without concomitant neuralgia.

NEUROLOGY

Changes in Cognitive Function and Physiological Tremor as Possible Indicators of Brain Injury Following Open Heart Surgery

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Introduction: The prevention of ischemic brain injury related to open heart surgery is now considered as an intensively researched area. The simultaneous examination of sensory and motoric mechanisms gives a reliable interpretation on the cerebral involvement. An accepted method in neurology for investigating cognitive funtions is the complex analysis of P200 and P300 evoked response potentials (ERP). The aim was to measure and analyse sRT and cRT values which correlate significantly with P200 and P300 potentials. We developed a multi-functional system with a software which is able to measure and record these two kind of reaction times and physiologic tremor during the observation in bed-side.

Material and Methods: We analyzed 123 patients (age: 31-71; 88 male, 35 female; 57 CABG, 26 valve replacement, 31 combined surgery, 9 Off-Pump) on the preoperativ day and one of the postoperativ 3-5th day. We compared the pre – and postoperativ sRT and cRT values (Mann-Whitney probe). The tremor measurement was performed with our equipment based on Analog Devices ADXL 320 JPC integrated accelerometer chip, and the digitized record was stored on a PC. FFT spectral analysis was performed on the tremor function: we compared the power integrals at the 1-4 Hz, 4-8 Hz, 8-12 Hz frequency ranges, and these power integrals were statistically assessed by Wilcoxon rank correlation test.

Results: Significant elongation was observed in both sRT and cRT in connection with certain periods of the operation ($p<0.05$). The power spectrum of physiologic tremor has changed, the spectrum at 8-12 Hz range (neuronal oscillation) has decreased and a shift was recognized to the lower spectrum ($p<0.01$).

Conclusion: The assessment of neurocognitive functions and physiologic tremor by our method may give a deeper insight into the pathophysiology of brain damage with open heart surgery. Our observations and this method may form the basis of the spreading of neuroprotectiv conception and the neuropsychological rehabilitation.

NEUROLOGY

Cholinergic and Tachykinergic Characterization of the Physiological Colonic Peristaltic Activity of Mice

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Introduction: In inflammatory bowel disease, colonic peristaltic activity is disturbed and tachykinin receptors are upregulated. The functional role of tachykinins in colonic peristalsis in mice however remains unclear. Therefore the aim of this study was to characterize neuronal conduction and investigate the functional role of tachykinins in in vitro colonic peristaltic activity in this species.

Material and Methods: Colonic peristaltic activity was assessed by quantifying the amplitude and interval of distension-induced pressure waves in proximal and distal colon segments using a modified Trendelenburg set-up. In preliminary experiments, peristaltic activity was characterized by incubation with hexamethonium, atropine and tetrodotoxin (TTX). A second series of experiments focused on the contribution of tachykinins to peristalsis by studying the cumulative effect of blockade of tachykinin NK3, NK2 and NK1 receptors with SR142801, nepadutant and RP67580 respectively.

Results: Gradual distension of the colon resulted in spontaneous and repetitive rhythmic pressure waves. The amplitude of these waves was significantly reduced by hexamethonium (100 μM) in the proximal (6.18±1.10 to 0.79±0.41 cmH2O, n=7) and distal (6.43±0.34 to 0.56±0.28 cmH2O, n=6) colon. Atropine (1 μM) also successfully reduced the amplitude (proximal 5.83±0.43 to 2.15±0.48 cmH2O, n=6; distal 5.46±0.51 to 2.98±0.89 cmH2O, n=5), as did TTX (1 μM) (proximal 4.18±0.62 to 0.21±0.11 cmH2O, n=10, no experiments were yet conducted distally).

The interval was abridged by hexamethonium in the distal colon (49.83±4.85 to 16.11±9.11 s; n=6) with a trend toward shortening proximally (p=0.07). Atropin had no effect. TTX shortened the interval in the proximal segment (55.30±3.68 to 9.17±5.38 s, n=10), no experiments were yet conducted distally.

SR142801 (0.1- 0.3 μM) had no effect on the amplitude or interval. Nepadutant (1 μM) showed a trend towards diminishing the amplitude in the proximal colon (p=0.08), but no effect was seen distally. Also there was no effect noticeable on the interval.

RP67580 (2 μM) significantly reduced both the amplitude (6.51±0.94 to 1.60±0.83 cmH2O, n=6) and interval (63.25±4.80 to 106.06±22.42 s, n=6) proximally without an effect distally.

Conclusion: In vitro colonic peristalsis in mice is almost entirely mediated by neuronal nicotinic neurotransmission. We could not demonstrate a role for the NK3 receptors, while the NK2 receptors might be implicated and the NK1 receptors appear to be involved.

NEUROLOGY

Nitric Oxide, Predictor Factor of Infarct Growth after Cerebrovascular Stroke

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Introduction: There is evidence that inflammatory and excitotoxic mechanisms mediate neurologic deterioration (ND) after cerebral stroke. Reactive oxygen species are important mediators of ischemic tissue injury. In this study investigated relation of Reactive oxygen species with outcome and volume the diffusion weighted image (DWI) lesion after acute cerebrovascular stroke.

Material and Methods: MRI was performed on admission (T0) and at 3 days (T1) in 189 patients with acute cerebrovascular infarction of <12 hours' duration. DWI lesion enlargement was calculated as the absolute difference between volumes on T0 and T1 of evolution. NIH Stroke Scale was scored at the same intervals. ND was defined as an increase >/=4 points within the 72 hours. Nitric oxide (NO) and Peroxynitrite (ONOO–) levels were analyzed in blood samples obtained on admission.

Results: DWI lesion growth was found in 136 (71%) patients (median increase 37 [6.5, 83.4] cm (2.9)) and ND occurred in 50 (26.4%) patients. Baseline NO ($r = 0.70$), ONOO ($r = -0.34$), showed a significant correlation with the DWI lesion growth (all $p < 0.001$). After adjustment for potential confounders, NO levels were the only Reactive oxygen species associated with DWI lesion enlargement at 3 days (beta = 0.20; SD = 0.06; $p = 0.003$).

Conclusion: Production of reactive oxygen species after cerebrovascular stroke may play a role as mediators of lesion enlargement in cerebral ischemia. Plasma NO concentration is the most powerful and independent predictor biomarker of lesion enlargement in the acute phase of cerebrovascular stroke.

NEUROLOGY

Neurological Complications after Eversion Carotid Endarterectomy

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Introduction: Stroke is identified as the third leading cause of death in Western world. Eversion carotid endarterectomy (ECEA) is well known method in the treatment of extracranial carotid stenosis in the ischemic stroke prevention. The aim of this retrospective study was to assess major and minor postoperative complications compared with that under standard operation.

Material and Methods: Retrospective analysis was performed of the hospital charts from 117 consecutive patients, subjected ECEA under regional anesthesia, between May and December 2002 in Vascular and General Surgery Department of Medical University of Silesia in County Hospital in Bytom. There were 29 female (24.8%) and 88 male (75.2%) with mean age 68.1 years (ranged from 50 to 86). Near occlusion stenosis was confirmed by CT angiography in 7 cases (6%). Over 70% stenosis of the internal carotid artery was the indication to the operation in both symptomatic and asymptomatic patients. 105 of ECEA were done for symptomatic disease. There were 23% amaurosis fugax, 66% TIA's and 37% strokes in symptomatic patients. Surgery for asymptmatic patients was performed before cardiac and major vascular procedures. All patients had a standard pre-operative and post-operative assessment performed by a neurologist.

Results: No major complications (death/stroke) were observed in postoperative period. There were 8 (6.8%) TIA's: two perioperative and 6 in first three hours after the operation. One cranial nerve injuries were observed. Four patients had injuries of the marginal mandibular branch of the facial nerve and one patient had lesion of the hypoglossal nerve. Six patients developed hoarseness without cranial nerve injury, recovered within one week in 4 cases. I compared this outcomes with results presented in literature. Neurological complications after standard carotid endarterectomy in centers of excellence is assessed less than 3% for major complications (death/stroke). In my study group I didn't find any stroke and death. This excellent outcome is significantly different. 6.8% TIA's in my material is significantly more common than in other authors(1-3%) Nerve injuries are reported form 2,6% to 5,6% in multicentre trials. In my study there were 4.3% such complications.

Conclusion: Carotid endarterectomy reduces the risk of stroke among patients with symptomatic and asymptomatic carotid stenosis. ECEA is technically more difficult but offers better postoperative outcome. It's hard to explain the high incidence of the TIA's in my material because operative time and cross-clamping time in this group was comparable with the others.

NEUROLOGY

Relation Between Different Types of Cerebral Injury and Cerebellar Volume in Very Preterm Infants

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Introduction: Injury to the developing cerebellum is an important complication of prematurity and can lead to abnormal cerebellar development and smaller cerebellar volume. The aim of this study was to evaluate the relation between different types of cerebellar injury and cerebellar volume in preterm infants around term-equivalent age. A secondary aim was to evaluate correlations between cerebellar volume and supratentorial brain injury and several clinical parameters.

Material and Methods: A cohort of 91 very preterm infants (gestational age < 32 weeks) was included. Sequential cerebral US scans during hospital admission and an MRI examination around term-equivalent age were performed in all infants. Cerebellar volumes were measured on MRI by manual segmentation. The correlation between cerebellar volume and different types of cerebellar and supratentorial injury on cerebral US scan and MRI and several clinical parameters was evaluated.

Results: Large cerebellar hemorrhages were significantly correlated with smaller cerebellar volumes ($p = .000$), but small punctate hemorrhages were not.

There was no correlation between different types of supratentorial brain injury, including white matter injury and intraventricular hemorrhage, and cerebellar volume. Infants with unilateral supratentorial brain injury, such as porencephalic cysts, periventricular hemorrhagic infarction and post hemorrhagic ventricular dilatation, did not have significant smaller cerebellar hemispheres on the contralateral side.

Low birth weight ($p = .014$), intrauterine growth retardation ($p = .004$), postnatal dexamethasone treatment ($p = .000$) and hypotension ($p = .002$) were significantly associated with smaller cerebellar volumes around term equivalent age. No correlation was found for gender, plurity, gestational age, antenatal steroid treatment, perinatal sepsis, respiratory distress syndrome, bronchopulmonary dysplasia and persistent ductus arteriosus.

Conclusion: Large hemorrhages in the cerebellum, of very preterm infants, were associated with a reduced cerebellar volume, around term equivalent age, in contrast to small punctate cerebellar hemorrhages.

No association was found between supratentorial brain injury and cerebellar volume, and there was no evidence for crossed cerebral cerebellar diaschizis.

Several clinical factors were identified as potential risk factors for smaller cerebellar volumes in preterm infants around term equivalent age. To evaluate the implications of these factors for cerebellar growth and development and neurodevelopmental outcome long term follow-up studies are needed.

NEUROLOGY

The Most Frequent Anomalies of the Arterial Cerebral Circle

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Introduction: The arterial cerebral circle is a crucial structure supplying the CNS. The anatomy of the cerebral arterial circle differs among individuals. The vessels of cerebral circulation may present changes resulting from external factors (age, diet, genetics) that may predispose to obstruction of one of the cerebral arteries. This is significant since in cases of a not fully intact circle there may be impaired blood flow due to lack of collateral circulation.

"AIM" The aim of study was to assess the incidence of the different anatomical variations in the Circle of Willis.

Materials & Methods: 180 preserved brain specimens were analyzed using a surgical microscope and microsurgical tools. Each specimen was first dissected of arachnoid mater. Then each major branch of the circle of Willis was examined in detail using the microscope. We made sure to exclude any cases presenting pathologies. We defined a vessel as hypoplastic if it was in 1:2 proportion to the opposite side or if its' diameter was less than 0.7 mm.

Results: The most common variation was a double anterior communicating artery (ACoA) found in 20% of specimens. Other variations of the ACoA were a triple trunk (1.6%), and a "Y" shape trunk (8.8%).The second most frequent variation was a triple A2 segment of the anterior cerebral artery (ACA) (11.1%). In 17/180 cases (9.4%) we found hypoplasia of the right posterior communicating artery (PCoA) and in 6/180 (3.3%) incidence in the left. In 2 of these cases the hypoplasia of PCoA occurred simultaneously on both sides. Hypoplasia also occurred in the A1 segment of right ACA (3.3%). Interestingly, we did not observe any hypoplasia of the left ACA. The fetal type division of the posterior communicating artery (PCA) was observed in 6% occurring more often on the left side (10:1). Statistic analysis showed that: single modifications were more often observed than numerous p=0,0000, variantion of the anterior part of circle (ACoA + A1 + A2) exist essentially more often than in posterior part (PCoA + PCA), p=0,0000, ACoA variation exists essentially more frequently than any other variation, p=0,0196.

Conclusion: The circle of Willis is a variable structure. Anomalies tend to be located on the right side and in the anterior circulation. The most often occurring anomaly of the left side is the formation of PCA from the internal carotid artery known as the "fetal" type.

NEUROLOGY

Significance of the Myosin Posphatase in Gutamatergic Nurotransmission in the Rat Ventral Ochlear Nucleus

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Introduction: So far, the possible regulatory roles of the myosin phosphatase have been studied in various contractile structures, mostly in the skeletal muscle. More recently, however, it has been demonstrated that this enzyme may also have roles in the synaptic neurotransmission. To reveal additional data about the possible functions of the myosin phosphatase, we studied the glutamatergic neurotransmission in one of the largest synapses of the central nervous system, termed the endbulb of Held, where the acoustic nerve terminals make contact with the cell bodies of the bushy cells.

Material and Methods: In the present work, 200 μm thick slices were prepared from the ventral cochlear nucleus of 9-12 days old rats, and the whole-cell configuration of the patch-clamp technique was utilised. To elicite excitatory postsynaptic currents (EPSCs), 10 repetitive stimuli (50 Hz) were delivered onto the acoustic nerve fibres, using a monopolar extracellular electrode.

A number of slices were preincubated in a solution containing 5 μM tautomycetin (a specific inhibitor of protein phosphatase 1).

Results: In these experiments, the amplitude of the first EPSC decreased, whereas the amplitude of the subsequent EPSCs showed relative or absolute increase when compared to the control situation. Moreover, the decay time constans (decay τ) of the EPSCs showed a nearly twofold increase. As opposed to these changes, when the slices were preincubated with the rho-kinase inhibitor Y27632 (10 μM), the amplitude of the first EPSC increased, whereas that of the subsequent EPSCs reduced. Moreover, the decay τ decreased by 50%. In experiments, where the combined application of the two inhibitors was attempted, Y27632 partially prevented the development of the tautomycetin induced effects.

Conclusion: The present results not only suggest that alteration of the myosin phosphatse activity influences glutamatergic synaptic transmission in the ventral cochlear nucleus, but they also indicate that this effect has both pre- and postsynaptic targets. This concusion was supported by the results of immunohistochemical experiments as well.

NEUROLOGY

Clinico-morphological Correlations of Remote Consequences in Patients with Traumatic Brain Injury

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Introduction: One of the main priorities of modern clinical neurology is the study of traumatic brain injury (TBI) and consequences which arise up in persons having a TBI. Aim of our work is studying of remote consequences of the TBI.

Material and Methods: We observed 40 patients aged from 4 to 16 years (31 male and 9 female) at the Neurosurgery Department of the Kharkiv City Emergency Hospital. In anamnesis 10 children had severe, 4 children – moderate and 26 mild severity of TBI (by A.N.Konovalov classification, 1998).

All the patients were given complex standard examination.

Investigation of archive material of seven 3-14 aged dead children got TBI in anamnesis (using complex of morphologic and morphometric methods) had been conducted for discovering clinico-morphological correlations.

Following syndroms have been manifested: syndrome of spastic paresis (2 children with severe TBI), ataxic syndrome (4 children with severe TBI), speech disturbances (5 children), dysarthria in 3 and stammering in 2 patients. In 15 children memory and attention had features of asthenic weakness (cerebroasthenic syndrome) in the form of productivity decreasing at loading. Intellect disturbances were detected in 3 children (7,5 %), delay of psychomotor development - in 1 child (2,5 %). MRI: hypotrophy of the cortex was in 8 (22,5%); porencephaly in 3 (7,5%), hydrocephaly in 2 children (5%); sclerosis of anterior and posterior parts of corpus callosum– in one child (2,5 %) and 29 patients (72,5 %) with mild and moderate extents of TBI in anamnesis had normal tomogram. Morphological: determined that neurons ruin is caused by neurophagocytosis by glial cells or by apoptosis. Neuronophagia of neurons, increasing of astrocytes activity are observed after concussion. In case of severe traumas oligodendroglia, microglia, microcirculation vessels, soft brain integuments are injured. Reparative angiogenesis (that is always accompanied by hyperangiomatosis) occurs during 6 month after injury and longer.

Results: In remote period of TBI the veritable increase in astrocytes average diameter is detected. This phenomenon indicates elevation of their morpho-functional activity especially expressed at severe traumas. Alterations of soft brain integuments represent productive arachnoiditis with formation of interintegument conjunctions. These phenomena results in liquorodynamics disturbance and hydrocephaly arising.

Conclusion: Alterations of brain are always of organic character in a remote period of TBI; it was demonstrated correlation between the extend of TBI, local alterations of brain and state of patients in remote period.

NEUROLOGY

A Relationship Exists Between Behavioral Activity and the Endogenous Biological Clock of theSuprachiasmatic Nuclei

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Introduction: All organisms show 24hr rhythms in behaviour, physiology and biochemistry which are therefore termed "circadian rhythms". If an organism is deprived of external cues such as daylight or temperature, these rhythms persist with a comparable period. The SCN (suprachiasmatic nuclei) in the anterior hypothalamus are responsible for these endogenous circadian rhythms.

Material and Methods: The SCN consist of ~20,000 neurons which together have a rhythm in action potential frequency, a major output of the SCN. This rhythm, otherwise termed multiple unit activity (MUA), can be measured in living mice by implanting an electrode in the SCN. The MUA reaches its highest point during the subjective day - the period when nocturnal animals such as mice are least active. The MUA reaches its lowest point during the subjective night - the period when mice are most active. A high amplitude of MUA is needed for healthy sleep-wake cycles. For example, the elderly and depressed people often have sleeping difficulties. They are more tired during the day, frequently needing naps, and they have difficulties falling and remaining asleep at night. It has been shown that the circadian amplitude is attenuated in these people.

Results and Conclusion: On a very different timescale, we show that brief episodes of behavioral activity are related to a decrease in MUA. This indicates that connections exist between behavioral centra and the SCN, whereas it was formerly thought that the SCN functioned independently. The MUA amplitude can be increased by bright light exposure during the day. As active mice behaviour is related to a decrease in MUA and mice display most activity during the night, these decreases may contribute to a higher MUA amplitude. The attenuated sleep-wake cycles of the elderly and depressed persons may therefore not only benefit from bright light exposure, but also from more active behavior during the day.

OBSTETRICS AND GYNAECOLOGY

The Road to Obstetric Fistula

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Introduction: The aim of this study is to describe the process of developing an obstetric fistula for women in Tanzania.

Material and Methods: A retrospective record review was conducted of all women who were seen with obstetric fistulas at CCBRT-rehabilitation hospital in Dar Es Salaam, Tanzania, from January 2007 to December 2007. Detailed information on antenatal care and delivery process was obtained by questionnaires in a subgroup seen from September 2007 to December 2007.

Results: Of 171 patients with fistula 167 fistulas (97,6%) were associated with labour and delivery. A significant shorter stature was associated with fistula patients having repair in Dar Es Salaam. A high rate of antenatal care coverage (95,2%), but wide variations in quality of provision of care was observed. Mean labour time was 2,1 days and reasons for inadequate obstetric care are a delay in seeking care (fase 1), reaching care (fase 2) and implementation (fase 3) of care. Forty three percent went to a hospital >12 h after the beginning of contractions, with mean travel time of 4,1 hour and long waiting times at health facilities. C/S was conducted in 50,6% of patients, of which in 81,7% C/S was performed too late and followed by stillbirth. With ≥6,6% ureteric fistulas, caesarean section itself may be regarded as a factor causing fistula.

Conclusion: The development of an obstetric fistula in Tanzania mainly depends on lack of access to good quality of antenatal and obstetric care combined with socio-economic factors, such as poor education, stunting (malnutrition) and early childbearing.

OBSTETRICS AND GYNAECOLOGY

Comparison of Mechanical Ventilation Regimen in Preterm Infants with Different Outcomes

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Introduction: To improve mortality and morbidity rate in preterm infants who were admitted to neonatal intensive care unit with respiratory disorders.

Material and Methods: The study was conducted in neonatology unit of Kharkov regional hospital. Retrospective analyses of total 27 preterm infants were included in the study. Out of 27 neonates 12 (44%) died and 15 (55%) survived categorized as 1 and 2 groups. The study was carried out focusing on maternal anamnesis (multiple pregnancies- MP, infection pathology- IP, pre-eclampsia- PE), character of delivery (caesarian section- CS, placenta abruption- PA) and fetal anamnesis data (hypoxic ischemic encephalopathy- HIE, intracranial hemorrhage- ICH, brain circulation insufficiency- BCI, brain edema- BE, severe asphyxia- SA) were postulated the results.

Results: The 1 group of mean birth weight 3000±840 with mean gestational age 24±37 with maternal anamnesis MP-26%, IP-33%, PE-26%. Character of delivery showed with CS- 53%, PA- 46%, and fetal anamnesis data HIE- 93%, SA- 60%, ICH-93%, BCI- 53%, BE-86% were revealed where as in 2 group of mean birth weight 2700±1000 with mean gestational age 28±37 with maternal anamnesis MP- 9%, IP- 36%, PE-36%. Character of delivery data CS- 45%, PA- 27%, and fetal anamnesis HIE- 81%, SA- 9%, ICH- 27%, BCI- 22%, BE- 36% were revealed. Ventilator settings varied with individual cases but generally the ventilator was set initially at a peak end expiratory pressure (PEEP) of 4-5 cm H2O, peak inspiratory pressure (PIP) of 18-20 cm H20, rate of 50/min and an Fi02 sufficient to maintain 02 saturation greater than 90%.

Conclusion: The judicial use of neonatal intensive care measures in a developing country can results in a reduction on morbidity and mortality.

Survival rates increased with increasing birth weight and gestational age and decreasing complications. The Multiple Pregnancy, Placental abruption, Severe Asphyxia, and Intracranial Hemorrhage are more frequently were in neonates with respiratory disorders who had unfavorable outcomes in our study and are estimated as death predictors. The focus should begin with obstetric measures for the prevention of preterm delivery, close monitoring of high risk pregnancies and judicious use of tocolytics.

OBSTETRICS AND GYNAECOLOGY

Breast Volume Determination in Breast Asymmetry Patients

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Introduction: It was always a sort of problem for plastic surgeons to determinate the volume of the breast before choosing an implant in treating breast asymmetry patients. Although there are quite reliable methods to do that most of them are expensive and complicated. So we think it is worth to have a simple and cheap method in everyone's practice. The aim of this study was to introduce a simple and reliable method to determinate the right volume and profile of the breast implant in breast asymmetry patients before breast augmentation.

Material and Methods: We used alginate impression material to seal 18 breasts of 10 patients with breast asymmetry (2 women after unilateral mastectomy, 1 patient with Poland syndrome, 1 patient with tuberous breast and 6 patients with asymmetric glandular hypotrophy). Filling the seals with water we determined the volume difference between two breasts. After that we made the models of the breasts by filling the seals with gypsum. Then we measured the differences of the heights between the models of two breasts. 10 patients with breast asymmetry underwent breast augmentation and evaluated their postoperative breasts asymmetry visually.

Results: Implants were chosen to match the volume and profile for each patient before augmentation. The mean difference of breast volumes was 72.14 ml; the mean difference of breast heights was 0.77 cm before augmentation and accordingly 13.75 ml and 0.41 cm after augmentation. All 10 patients were satisfied with the overall result of the operation. Still 2 of them stated that their breasts were slightly asymmetric in volume (mathematically the differences were 25 and 30 ml) and one noted her breast being of different heights (mathematically – 1.2 cm difference). None of them said these differences to be esthetically significant.

Conclusion: Breast volume determination using alginate impression material is a simple and reliable method for plastic surgeons to evaluate breast asymmetry objectively, to determinate the difference of breasts volumes and heights and to choose the implant(s) of the right volume and profile for the patient.

OBSTETRICS AND GYNAECOLOGY

Learning Curve for Fetoscopic Laser Coagulation

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Introduction: The aim of this study was to make an estimation of the learning curve for fetoscopic laser surgery for twin-to-twin transfusion syndrome (TTTS).

Material and Methods: Retrospective study on 176 consecutive monochorionic twin pregnancies with severe mid-trimester TTTS treated by fetoscopic laser coagulation between August 2000 and December 2006. The procedures were at first performed by two operators, later joined by another two operators, commonly working in teams of at least two. Learning effect was assessed by comparing outcome of the first 50 cases with the next 126, and by comparing cumulative survival rates for each operator over time. Primary outcome was neonatal survival at 4 weeks of age.

Results: We found an almost linear relationship between the probability of fetal survival and the number of procedures performed by the operator. At least one survivor occurred in 80% in the first 50 pregnancies and 84% in the subsequent 126. The percentage of pregnancies with survival of both fetuses increased from 52% to 58% Cumulative survival rate for each operator showed no significant differences between the operators.

Conclusion: This study shows increasing survival rates with growing experience in fetoscopic laser coagulation technique. No significant difference in learning curves of four operators for fetoscopic laser coagulation for TTTS was found. Results of our center working with four operators showed similar pregnancy outcomes as in published series with one operator. The presumed disadvantage of dilution of procedures does not seem to exist in our unit, possibly due to the team-approach.

OBSTETRICS AND GYNAECOLOGY

Maternal Care in Health Centres in Thyolo District, Malawi

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Introduction: District health managers and health workers in Thyolo district intend to combat the high maternal mortality figures in the district. The aim of this study is to provide them with a situational analysis of maternal care in health centres (HCs), balancing the burden against the available resources.

Material and Methods: In thirteen HCs and in Thyolo District Hospital (TDH) information on admissions, deliveries, referrals and human and material resources was collected.

Results: In the Thyolo HCs 6274 deliveries were counted in 2007. Of all expected pregnant women in the district, it was estimated that 44.9% delivered in a health facility. Of women admitted in a HC, 11.3% (range: 2.4-24.0%) ended up being referred to the district hospital. The main reason for referral was prolonged labour. In TDH, the number of expected obstetric complications treated increased between 2006 and 2007.The average burden per midwife was six deliveries a week (range: 2-13). The four HCs with the highest number of referrals had more than six deliveries per midwife per week. Drugs and labour utensils were often found to be out of stock. Of all women who sustained severe complications of labour, 92% went for at least one antenatal visit. Only 46.6% had their blood pressure checked at least once during the pregnancy. After requesting for an ambulance from the hospital, it took an average of three hours for the ambulance to reach the HC (range: 20 minutes-6 hours).

Conclusion: The quality of care in HCs in Thyolo district deserves urgent attention. HCs require more and better-managed human resources to reduce the workload per midwife. Material resources need to be managed more efficiently in order to avoid stock ruptures. ANC should be performed according to WHO-standards. The referral system should be made more efficient and effective by improving the transport system from the HCs to TDH. Referrals should be performed according to clear guidelines. These recommendations will be communicated to the relevant authorities in Thyolo district.

ONCOLOGY

CTLA-4 Gene Polymorphism in Patients with Bladder Cancer, Shiraz, South of Iran

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Introduction: Bladder cancer is the most common malignancy affecting urinary system. After formation of malignancy , tumor cells express an abundance of self and foreign antigens which immune system must eliminate them. Cytotoxic-T-Lymphocyt-Associated protein 4 (CTLA4) is a negative co-stimulator molecule which is expressed on the surface of activated Tcells. It plays a role in terminating T cell responses and in self tolerance. In this study we tried to evaluate any relationship between different polymorphism of CTLA4 gene and risk of bladder Cancer.

Material and Methods: In this cae-control study, the case group was selected from 113 patients with confirmed pathological report of bladder cancer in different types and grade.The demographic data were collected from each patient in a separate questionnaire .Also 113 age and sex healthy individuals were enrolled to the study as control group

From each participant in both case and control groups 10cc of whole blood was taken and stored at -20c in an EDTA tube for DNA extraction. CTLA4 exon 1 position 49 polymorphism was investigated by PCR-RFLP method
The polymorphism in case and control groups was compared in regards to the age of onset, history of smoking, sex, tumor site, tumor grade, local and distant metastasis using chi-square, kruskal wallis and fisher's exact test .Differences were considered to be statically significant if the p value was less than 0.05.

Results: CTLA4 gene polymorphism in the case (85% male) and control group (75% male) were as follows: AA , AG, GG genotype in the case group 57.5%, 37.2%, 5.3% respectively and in the ctrl group 57.5%, 32.7% and 9.7% And there was no statically difference in case and ctrl group ($p \geq 0.05$). Cancer characteristics like mean age of onset of cancer, tumor size and gene polymorphism were compared, which was shown no significant correlation in this regard. Association of tumor grade and distant metastasis with and gene polymorphism was evaluated which revealed no significant relationship.

Conclusion: The showed that CTLA4 gene polymorphism is not associated with genetic susceptibility to bladder cancer in studied population from Iran. The defect in immune system for suitable response for a an antigen can be attributed to the different steps like presentation of the antigen, Identification of the antigen, activation and proliferation of the immune components and.... For this reason the defect may be in each parts of those stages.

ONCOLOGY

The Role of SHREW-1 Gene in Human Glioblastoma Multiforme Cells

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Introduction: I performed series of experiments to monitor change in proliferation, migration, morphology and adhesion in human glioblastoma multiforme (GBM) cell lines due to SHREW-1 gene knock-down.Tumors of the central nervous system are the second commonest tumors in children and the sixth commonest in adults. GBM accounts for 30% of all primary tumors in adults. Survival is usually less than a year. Shrew-1 is a novel membrane protein involved in adherens junctions. It is not expressed in about 20% of human glioblastoma multiforme tumors. The suppression of the gene may change clinically relevant cell behavior.

Material and Methods: SHREW-1 was knocked down in U-251 cell line using RNA interference and confirmed by cDNA synthesis and PCR. Proliferation was measured using MTT Proliferation Assay in five-day time course, cell migration was measured using Wound Assay and Transwell Migration Assay toward Growth Factor in six- and twelve-hour time course, cell adhesion was measured on laminin coated wells.

Results: The proliferation rate of U-251 cell line was lower in SHREW-1 knock-down (KD) cells compared to control. The average proliferation ratio of control cells compared with KD cells is 1,56x higher in five-days time course. The transwell migration study showed 1,83x higher average ratio of cell migration after 6 hours in KD cells compared to control. In 12 hours, the average ratio of cell migration would be 1,23x higher in KD cells compared to control. The Wound Assay showed that KD cells do not heal within 48 hours while control cells fully healed at this time point. The adhesion to laminin of KD cells is 1,23x higher compared to control. The SHREW-1 knock-down cells from long branching processes 16 days after transfection and have apparent difficulty conforming a monolayer if knocked-down at lower confluency compared to control cells.

Conclusion: Shrew-1 loss decreases proliferation of U-251 cells, it enhances migration in transwells, however it decreases migration in Wound Assay, probably due to loss of cell polarity and possibly change in proliferation. It also changes the adhesion of the cells to extracellular matrix protein – the KD cells are more prone to adhere to laminin coated wells. Shrew-1 loss also alters morphology of the cells – their branching, shape, length of processes.

ONCOLOGY

Influence of Doxorubicin on Cell Death and Antioxidant System in NK/Ly Lymphoma Cells in Mice

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Introduction: Many efforts have been made to understand the role of ROS in cascade metabolic changes that lead to tumor cell transformation. More precise attention has been paid to the role of ROS in the metabolism of the tumor cells under the influence of cytotoxic remedies as their influence is often crucial in the development of chemoresistant tumor cells.

Material and Methods: NK/Ly lymphoma was modeled in 15 healthy mice. Ascite liquid drainage was made on the 10th day after the tumor cells introduction. Experimental animals were divided into two groups – untreated mice and mice treated with doxorubicin in the dose of 1 mg/kg with 1 day interval starting from the third day of experiment.

Morphological analysis determined total amount of tumor cells and their mean volume. Metabolic status of the cells was also estimated according to following parameters: middle mass molecules, concentration of NOx, malone dyaldehide and derivatives of proteins oxidative modification, activities of catalase and SOD activity.

Results: Total amount of tumor cells was decreased to 4.8±0.19 108/ml (control 7.6±0.11 108/ml), but their mean volume was increased to 95.69±6.89 µl3 (control 45.11±2.48 108/ µl3). Concentration of malone dyaldehide was decreased to 1.15±0.037 µmole/g of protein (control 1.69±0.021 µmole/g of protein) in ascite plasm, concentrations of derivatives of proteins oxidative modification in ascite plasm and middle mass molecules cell lysate were increased to 2.76±0.017 OOG/mg of protein (control 2.71±0.013 OOG/mg of protein) and to 13.78±0.031 OOG/mg of protein (control 6.82±0.025 OOG/mg of protein). Catalase activity was increased to 1.43±0.041 µmole H2O2×min/mg (control 1.01±0.019 µmole H2O2×min/mg) cell lysate, but SOD activity was decreased to 85±2.59 µmole NST/hv mg of protein (control 132±1.45 µmole NST/hv mg of protein) cell lysate. Concentration of NOx in ascite plasm and in cell lysate were decreased to 1.05±0.011 µmole/g of protein (control 1.51±0.016 µmole/g of protein) and to 1.99±0.017 µmole/g of protein (control 2.25±0.013 µmole/g of protein).

Conclusion: Cytostatic effect of doxorubicin was accompanied by reliable increase in mean volume of tumor cells and decrease in their total amount ($p<0.05$), which can testify apoptic, necrotic and hybrid types of cell death. Increase of intracellular level of middle mass molecules under the influence of doxorubicin testifies deep destructive changes in protein metabolism, which can be a start point of the endogen intoxication development during the treatment with cytostatic agents. Increased catalase activity may testify important role in regulation of cell apoptic process.

ONCOLOGY

Inflammatory Process Mediators and Staging of Colorectal Cancer

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Introduction: Colorectal cancer is the 2nd most common malignant tumor in European Union countries both in men and women. Colorectal cancer is also second cause of death from malignant diseases. Nowadays, apart from CEA level in blood as a perioperative prognostic factor, there is no other commonly performed laboratory test able to predict stage of colorectal cancer. Main objective of the study is to detect and estimate relations between routine blood tests with stage of disease.

Material and Methods: The study was performed on the group of 490 patients treated in Surgical Oncology Department of Medical University in Gdańsk, Poland. The group consisted of 278 men and 212 women. Average age was 64.5 (range: 23-90). Disease advancement according to AJCC classification was defined as stage II and III in 75% off all resectable tumors. Correlation between demographic and clinical data (age, TNM and AJCC status, localization and differentiation grade of tumor, number of resected and metastatic regional lymph nodes) and value of blood test (HGB, RBC, HCT, PLT, WBC, GRAN, %GRAN, LIMF, %LIMF, MONO, %MONO,EOS, %EOS, APTT, PT, INR) was calculated.

Results: Hemoglobin level was related to localization of primary tumor in the ascending colon ($p=0.0001$). Correlation between granulocyte level and features T, N and stage in AJCC classification ($p<0.005$; $p<0.05$; $p=0.001$) was found. Analysis revealed also high correlation between granulocyte/lymphocyte ratio and tumor size ($p<0.05$). No statistical significance between mediators level in serum and factors as: age, differentiation grade of tumor, number of resected and metastatic regional lymph nodes was found.

Conclusion: High correlation between stage of disease (AJCC, TNM), level of granulocyte and granulocyte-lymphocyte ratio emphasizes the meaning of the inflammatory process in tumor growth. The results show directly the exploring character of the study. In supplementary part additional mediators of inflammatory process (CRP, TNF-alpha, IL-1, IL-6) will be included into consecutive analysis.

ONCOLOGY

Impact of *Helicobacter pylori* babA2 on Gastric Cancer in South Africa

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Introduction: Infection with *Helicobacter pylori* is associated with different human gastric diseases. Although the prevalence of the infection worldwide is very high, only 10-20% of the carriers develop gastric diseases. In Africa there is a discrepancy between the infection-rate and the incidence of gastric diseases. This phenomenon called 'African Enigma'. In contrast with western countries where the prevalence is low but the incidence of gastric cancer relatively high. The clinical outcome is determined by a complex interaction of environmental influences, host, microbial virulence factors. The outer-membrane-bound protein, babA2, is an adhesin of *H. pylori*, interacting with the antigen Lewisb on epithelial cells. The adherence seems to be fundamental for the pathogenicity of the bacterium. It is speculated that patients harbouring the babA2 genotype have a higher chance to develop severe gastric disease.

AIM: The purpose was to ascertain the prevalence of the babA2 genotype in dyspepsia-patients in South Africa and compare the prevalence in the low-and high-risk group.

Material and Methods: Forty-nine patients, who were admitted to the hospital for dyspeptic complains, were enrolled in the study. Six biopsies from the antrum and corpus were obtained from the patients. Four biopsies were used for DNA extraction. The other two were histologically evaluated for neutrophil polymorph activity, helicobacter density, chronic inflammation, atrophy and intestinal metaplasia.

Results: Thirty of the 46 patients were positive for *H. pylori*. A total of 11 infected patients harbored babA2. A prevalence of 36,67% was examined in the individuals from South Africa. Although a significant difference in *H. pylori* observed between the black and white population, none was found comparing babA2. Increased neutrophil activity in the antrum was noted in the patients positive for babA2 ($p = 0.009$). In the corpus the activity did not reach a significant point. Furthermore the helicobacter density in the patients carrying a babA2 strain was significant higher in the corpus ($p = 0,045$) in contrast with the results found in the antrum. As expected atrophy and IM was more frequent in the babA2 positive group, the only case detected with IM was found in a babA2 positive patient.

Conclusion: Looking at other studies the prevalence found in the current study is relatively low. Although some of these data suggest a relationship between carrying a babA2 strain and the pathogenesis of severe histological changes, this have to be explored in a larger study.

ONCOLOGY

Left Inguino-Scrotal Hernia Associated with Synchronus Colonic and Colorectal Cancer

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Introduction: The simultaneous occurrence of inguinal hernia and colorectal cancer is rare and has a major impact on further surgical therapeutic plan.

Material and Methods: A 77-year-old man with a large inguinal left hernia, with usual preoperative investigations in normal range and no clinical signs or symptoms for digestive malignancy, underwent surgical procedure for hernia repair. Through a left inguinal incision, a large hernia sac was delivered from the scrotum. The sac contained a distended segment of colon that was partially obstructed by a carcinoma. At median laparotomy a second synchronous lesion was discovered. Left colorectal resection with end-to-end anastomosis and polypropylene mesh hernia repair was performed.

Results: The hospital course was uneventful.

Conclusion: This is an illustrative rare case of intrasaccular tumor of the colon, also associated with synchronous colorectal cancer. In such cases the therapeutic plan is changed and two options are available: simultaneous treatment or the delayed resolution. The decision is based on surgical team experience and particularity of each case.

ONCOLOGY

Chemoprevention of Cutaneous Malignant Melanoma

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Introduction: Primary prevention strategies of cutaneous malignant melanoma (MM) have limited effectiveness, as the incidence and the mortality in Caucasian populations, respectively 3 to 7% per year and 20% at 5 years, have increased more rapidly than those of other common cancers. Skin carcinogenesis and epidemiological studies suggest a new prevention strategy, namely chemoprevention, defined as the use of medications to prevent development of malignancy.

The objective is to explore the association between the use of NSAIDs and statins, and the risk of developing MM. The anti-tumoractivity and anti-inflammatory approaches of NSAIDs are due to the reduction of prostaglandins by COX1/COX2-enzyme inhibition. Statins reduce the enzyme HMG-CoA reductase and so the prenylation of Ras- and Rho-proteins, which is important in the carcinogenesis-pathway.

Material and Methods: In this multicenter case-control study, cases include patients of the department of dermatology of the universities of Antwerp and Louvain with a pathologically verified diagnosis of MM. For each case, two randomly selected control subjects are included. Based on a standardized questionnaire, we assess demographic characteristics, skin cancer risk factors, access to medical care and personal medical history including self-reported drug exposure through telephone interviews. To estimate the odds ratios with 95% CI and to adjust for confounding variables, a multivariate logistic regression analysis is used.

Results: 158 patients with MM and 424 controls are included in the study. After performing Chi square analysis, no significant difference is found with the use of NSAIDs and statins in those with MM compared to controls. Adjusting the 10 most important confounding variables in a multivariate logistic regression shows a 50% protective effect with the use of statins. On the contrary, the use of NSAIDs results in a 50% higher risk on development of MM, where the use of acetaminophen also shows a protective effect of 50%. However, these associations aren't significant and probably prone to coincidence, because the magnitude of data calculated by power analysis isn't yet collected. Nevertheless, this study can be complementary to a meta-analysis of a randomized control study for further exploration of these associations.

Conclusion: These results suggest a possible protective effect of statins and acetaminophen, while NSAIDs could be associated with a higher risk of developing a MM. An integrated approach combining primary prevention and secondary chemoprevention can be a new possibility to maximize effectiveness in reducing incidence rates of MM.

ONCOLOGY

Does Gamma Knife Radiosurgery Stop Vestibular Schwannoma Growth? A Prospective Study

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Introduction: Vestibular schwannoma (VS) is a benign tumor that arises in the eighth cranial nerve, giving symptoms of hearing loss, tinnitus, and vertigo. In general, it is a slow-growing tumor that in some cases even shows spontaneous regression. When and how to treat is debated, but in some centers, newly diagnosed small tumors are subject to conservative treatment, i.e. serial MRI scans without active treatment. In cases where the tumor has shown rapid growth in serial scans, or has become relatively large, treatment is usually offered. Two primary treatment methods prevail: microsurgery, where the tumor is physically removed; and gamma-knife radiosurgery (GKRS), where a gamma ray radiation dose is delivered to the tumor. This radiation dose, in theory, causes tumor growth arrest. In this study we wish to detail the success rate of GKRS, by comparing growth rates before and after GKRS treatment, and finding the proportion of tumors that get a successful treatment (defined as a negative or zero post-treatment growth rate).

Material and Methods: Between 2000 and 2006, 347 patients were diagnosed with VS. From these, 159 (46%) received treatment by the end of 2007, while the remaining 190 (54%) were treated conservatively. A total of 41 (22%) of these conservatively treated patients later received GKRS treatment due to growth of the tumor, as detected by the serial MRI scans. These 41 patients were included in the study, and were followed for a minimum of two years after treatment. The tumor volume on both pre-treatment and post-treatment images were measured, and mixed effects models were used to analyze the growth rates before and after treatment. We also conducted a logistic regression analysis to determine whether the age of the patient or the growth rate before treatment are related to achieving successful treatment.

Results and Conclusion: We found a lower success rate for GKRS treatment of VS than previously reported. This finding is likely because other studies have included patients that had no growth potential, while our study includes only patients who have had previously documented growth. Details will be presented at the conference.

ONCOLOGY

Quantitative Classification of Lymph Node Metastasis in Gastric Carcinoma – New Proposals Need to Overcome Current Classification?

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Introduction: In 1997 the TNM staging system introduced the quantitative lymph node metastasis classification (pN). Several authors have been testing new cut-off values to determine new categories for pN. This study was designed to assess the clinical applicability of a simple classification system based on the lymph node metastasis analysis of the cases of a single institution.

Materials & Methods: In this retrospective study we reviewed 522 cases of gastric carcinoma patients, who underwent resection surgery between 1982 and 2006, at the Department of General Surgery, S. João Hospital. According to the percentiles of the lymph node metastasis distribution, three groups of pN were considered: N0 – no regional lymph node metastasis, N1 – metastasis in 1 to 7 lymph nodes, and N2 – metastasis in more than 7 lymph nodes. All three groups were studied regarding clinical and pathological parameters, and cumulative survival. This classification system was tested, performing statistical multivariable analysis, according to the Cox regression model, with other known lymph node metastasis classification systems. $p<0.05$ values were considered statistically significant.

Results: In this study, 206 patients (39.5%) were classified as N0, 197 (37.2%) as N1 and 122 (23.4%) as N2. When comparing the distribution of the three groups: statistical difference was found in tumor location ($p<0.001$), macroscopic presentation ($p<0.001$), gastric wall invasion ($p<0.001$), WHO classification ($p<0.001$), Laurén classification ($p<0.001$) and Ming classification ($p<0.001$). We also found significant differences in the survival curves between the three groups. According to the Cox regression model, only the classification of the TNM staging system was considered an independent predictor of survival.

Conclusion: Despite not affecting the prognosis, the model proposed in this study confirms the importance of a quantitative evaluation of the LNM in gastric carcinoma. New models of quantitative lymph node metastasis classification should be evaluated.

ONCOLOGY

Expression of HER2 Protein in Infiltrating Ductal Carcinoma of the Breast.

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Introduction: The goal of this study was to determine the frequency of expression of HER2 protein infiltrative ductal breast carcinoma, and to establish the correlation between the expression of HER2 protein and standard prognostic factors of breast carcinoma.

Material and Methods: The research has been done on 100 randomly selected patients with infiltrative ductal breast carcinoma that had a mastectomy or lumpectomy performed coupled with the evacuation of axillar contents. The patients have been divided in to groups corresponding to the existence of metastasis in regional lymph nods at the time of the surgery, size of tumors, histological gradients of the tumor and HER2 status. The values of the measurements of tumor size, with the use TNM system of classification from the year 2000, have been taken from pathohistologycal records. The data regarding the degree histological differentiation of the tumor, done by using the Bloom-Richardson scoring system, by witch all tumors are divided in to good, medium and poorly differentiated carcinomas, and the status of regional lymph nods. Determination of the expression of HER2 protein in tumor tissue was done using the DAKO Hercept testa Kit K 5204, with results being interpreted using the appropriate scoring system.

Results: The increase of the expression of HER2 protein is found in 20% of the cases, distributed in the following order 4 out of 52 T1 tumors (8%), 11 out of 37 T2 cases (30%), 3 out of 6 T3 cases (50%) and 2 out of 5 T4 carcinomas (40%), ($p<0,05$ chi-square test). It has also been found that the tumors with positive HER2 expression are mostly larger then tumors with negative HER2 expression. The increased expression of HER2 protein has been found in 7 out of 17 grade III tumors (41%) and 13 out of 61 grade II tumors (21%) and has not been found among well differentiated tumors of grade I. The correlation between the expression of HER2 protein and the gradus of the tumor positive of a high degree and highly significant ($r =0,36$, $p<0,01$).

Conclusion: HER2 status is a significant prognostic and predictive factor for breast carcinomas. This study has shown a significantly greater incidence of expression Her-2/neu protein in tumors of a larger diameter and lower degree of histological differentiation.

ONCOLOGY

Trends in Palliative Treatment of Oesophageal Cancer

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Introduction: Oesophageal cancer is still a hard challenge for today's medicine. Late diagnosis makes it incurable in more than 95% worldwide. In this situation importance of palliative treatment of oespohageal cancer is beeing increasingly appreciated.

Material and Methods: The study was performed on 174 palliative patients with esophageal cancer operated in Surgical Oncology Department of Medical University in Gdańsk (1992-2007) operated in general (subgroup 1; n= 103) or local (subgroup 2; n=71) anesthesia. Our Department is a leading center of operative management of esophageal cancer, delivering treatment to 28% (16% - 43%/year) of all patients with diagnosed disease (1992-2007). Data was obtained retrospectively. T-student correlation test was used for the statistical analysis.

Results: Malnutrition and TNM status was similar in both groups The positive trend was observed in the number of palliative surgery procedures (range 5-23), with constant morbidity in the region (91-113/year; mean 98). Number of laparotomies performed in local anesthesia decreased from 60% to 9%, due to earlier delivery of the treatment. The survival of patients after palliative surgery was significantly longer in the subgroup with general anesthesia ($p<0.002$); and in the group with palliative surgery ($p<0.005$) compared with mortality of not operated patients in the region. The hospital mortality ($p=0.03$) as well as complication rate ($p=0.04$) was also lower in subgroup 1.

Conclusion: Our data confirm that early delivery of palliative surgery to the patients with oesophageal cancer increases survival, reduces stress related to local anesthesia during laparotomy and increases quality of life.

OPHTHALMOLOGY AND DERMATOLOGY

Chromosomal 6 Aberrations in Uveal Melanoma and an Inflammatory Phenotype

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Introduction: Uveal melanoma is one of the most frequently occurring primary intraocular malignancies in the Western world. Current evidence suggests that development of uveal melanoma progresses down one of two pathogenetic pathways, one involving the loss of chromosome 3 and the other involving chromosome 6. Chromosome 3 monosomy is detected in approximately half of uveal melanomas, is associated with an inflammatory phenotype, and is strongly linked to metastatic disease and decreased survival of the patient. The pathway beginning with chromosome 6 occurs in about one quarter of uveal melanomas and is known to be associated with a better prognosis. The hypothesis of this study is that the chromosome 6 pathway is not associated with an inflammatory phenotype.

Material and Methods: Tumor tissue was obtained from 50 cases of uveal melanoma treated between 1999 and 2004. After enucleation, chromosome analysis was performed by classical karyotyping on cultured tumor cells. In addition, nuclei were isolated from paraffin-embedded tissue for fluorescence in situ hybridization, to determine the chromosome 3 copy number. Each tumor-containing globe was further processed for conventional histopathologic examination and for immunohistochemical analysis with HLA class I and II-specific antibodies and with macrophage marker CD68.

Results: Of 50 uveal melanomas, 16% (8/50) were categorized as having an aberration on chromosome 6, where an aberration is defined as either loss or gain of genetic material. Chromosomal 6 aberrations were associated with a decreased density of tumor-infiltrating macrophages ($P = 0.008$) and a lower incidence of metastases ($P = 0.002$). HLA class I and II expression is lower in the patients with chromosomal 6 aberrations compared to the patients with monosomy 3, but the difference was not statistically significant ($P = 0.153$ and $P = 0.131$ respectively).

Conclusion: Chromosomal 6 aberrations in uveal melanoma are not associated with an inflammatory phenotype.

OPHTHAMOLOGY AND DERMATOLOGY

Creation of a Retinoblastoma Tumor Cell Vaccine

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Introduction: Retinoblastoma is the most common primary intraocular tumor in children and if left untreated, the tumor is universally fatal. Current therapies (radiation and/or chemotherapy) are succ sessful in many cases. However, there are difficult cases in which the current treatments control tumor growth at the expense of vision and/or significant toxicity, such as hearing loss. Therefore, new therapies are needed with increased tumor specificity and decreased toxicity.

Our long-term goal is to induce protective immunity in retinoblastoma (Rb) patients that selectively eliminates tumor cells without damaging the surrounding normal retina. We hypothesize that a CD80 / Class II positive Rb tumor cell vaccine will activate tumor-specific CD4+ T cells that sustain potent anti-tumor immunity. However, a tumor cell vaccine can only be constructed from Rb cells that do not express Class II or invariant chain. Genetically engineering these tumor cells to express only Class II (and no invariant chain) results in expression of cell surface Class II loaded with endogenous tumor antigens. Tumor-specific CD4+ T cells are activated when they recognize these antigens along with the CD80 costimulatory signal.

Material and Methods: Rb gene mutations were identified by sequencing in the cell lines studied (Rb107, 116, 125, 143). Class II and Ii chain detected via Westerns and flow cytometry. Rb cells were stimulated with recombinant IFN-g and tumor cells were transfected (AMAXA) with vectors containing cDNA for: Class II (DR1alpha and DR1 beta) and CD80.

Results: Two of the four Rb cell lines tested (Rb143 and Rb107) were candidates for a CD80 / Class II tumor cell vaccine and did not express either: (i) Class II (DR, DP, or DQ), or (ii) invariant chain. Moreover, stimulation of either Rb143, or Rb107 with IFN-g (up to 1,000 units) failed to trigger expression of Class II or invariant chain. By contrast, Rb116 constitutively expressed Class II (DR, DP, and DQ) and Rb125 expressed Class II (DR,DP, DQ) only when stimulated with IFN-g. Rb143 and Rb107 were transfected successfully with vectors containing the cDNA for: (i) Class II DR1 alpha and DR1 beta, and/or (ii) CD80.

Conclusion: We constructed successfully a Class II / CD80 positive Rb tumor cell vaccine using Rb tumor cell lines that do not express invariant chain. These results imply that Rb tumor cells can be genetically engineered to express endogenous tumor antigens via Class II that activate CD4+ T cells.

OPHTHAMOLOGY AND DERMATOLOGY

Evolution of 3-D atlas of Human Cornea with Age

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Introduction: The evolution of more sophisticated imaging technologies makes it possible to study the properties of human cornea. Despite the fact that eyes are an object to many research works, the human cornea still remains barely described.
Aim: The purpose of this study is to investigate age-related difference in the corneal topography of a normal population using a computerized corneal topography system.

Material and Methods: Human cornea was studied by Bausch & Lomb OrbSCAN technology. Patients were selected according to common criteria of inclusion among visitors of the Maisonneuve-Rosemont hospital in Montreal, Canada in the period 2000-2008. Patients have undergone a medical ophthalmological exam and afterwards a topographical scanner. Both men and women from different age groups were selected to participate in study, patients with glaucoma or eye surgery have been excluded. Data from patients` topographic corneal images is gathered and encoded. Every patient's image receives a code number to preserve confidentiality of the patient.

Results: The data from the corneal topographic images was statistically checked and summarized. The final result is a merged database containing data about the common parameters of a normal human cornea. Our ultimate aim is to create a precise atlas of the topography of human cornea.

Conclusion: Our results may serve as a basical atlas of the properties of the human cornea. More work on the anomalies of the human cornea, for example keratoconus, as well as studies on the varieties on human cornea in different ethnical groups and age groups may be the future directions in this field.

OPHTHAMOLOGY AND DERMATOLOGY

Local Injection of Bevacizumab (avastin) for Treatment of Primary Active Pterygium: A Prospective Randomized Clinical Study

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Introduction: Pterygium is a condition characterized by epithelial overgrowth of the cornea and occupying a nasal interpalpebral location. It has been shown that angiogenesis plays an important role in the pathogenesis of pterygia. As endothelial growth factor (VEGF) family is of the molecules which has well-known roles in angiogenesis, recently it was hypothesized that the recombinant humanized monoclonal antibody directed against VEGF (Bevacizumab, Auastin) can be a potential agent in the management of pteryyia. In this study we investigated the efficacy of local injection of bevalizumad for treatment of primary active pteryyium.

Material and Methods:Two hundred patients (200 eyes) with definite diagnosis of primary active pterygium without any other ocular problem participated in this Prospective, open labele, randomized clinical study at Poostchi clinic affiliated to the Shiraz University of Medical Sciences, Shiraz, Iran, participated in this study. The patients were allocated randomly in two groups to receive local injection of Bevacizumab (Avastin) (group 1) or normal saline as placebo (group 2) in the site of their lesions. We evaluated them with scoring to their signs and symptoms including : photophobia, conjuctival congestion and size of pterygium at 48 hourses ,then weekly up to one month and then monthly till to six months after injection. The level of statistical significance was set at $P< 0.05$.

Results: In the first 48 hours, conjunctival congestion was higher in group 1 than in group 2 (P: 0.00) as well as Photophobia (PV: 0.00) .There was no difference in size of pterygium. (P: 0.13) After one week, conjunctival congestion and photophobia was higher in group 1 than in group 2 (P:0.01) and (P:0.00) respectively ; however , the mean size of pterygium was not different between both groups (P :0.12).This trend was continued till 6 months after injection ,the time the results came back to be similar to first 48 hours after injection.

Conclusion: Local injection of Bevacizumab can be effective in improvement of primary active pterygium signs and symtoms; however, reinjection should be considered after six moths.

ORTHOPAEDICS

Assessment of Ossification and Fusion Level Following Anterior Cervical Discectomy with Interbody Cervical Spacers

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Introduction: Difficulties connected with operational treatment of cervical symptomatic degenerative disc disease (SDDD) arise from high risk of long term complications in the form of progressive degeneration of adjacent segments and microinstability as a result of collapsed intervertebral spaces and low rate bone fusion. In order to avoid symptom relapse restoration of the vertebral motor segment are a prerequisite. Techniques such as isolated discectomy and discectomy with autologus bone graft rely on the natural ability of bone tissue to regenerate and prevent the collapse of intervertebral space in a minimal degree, whereas implantation of cage systems with a central canal for osteoinductive materials both induce fusion to occur and keep the motor segment stable. The aim of the study was to assess the efficacy and dynamics of bone fusion in the operated segment of the cervical spine using anterior cervical discectomy (ACD) with an intervertebral cervical spacer and compare the results to ACD fusion (ACDF) with autograft from the ala of illium.

Material and Methods: 18 patients operated for SDDD in the cervical segment of the vertebral column were enrolled in the study. 4 female (mean age 55.5) and 5 male (mean age 52.6) underwent ACDF using an intravertebral spacer. 6 female (mean age 53.83) and 3 male (mean age 54.67) underwent ACDF using autograft from the ala of illium formed the control group. Clinical tests: Visual Analog Scale (VAS) and Neck Disability Index (NDI). For fusion and progressive degeneration of adjacent segments assessment Computed Tomography (CT) scans, Single Photon Emission Computed Tomography (SPECT) and planar scyntygraphy were performed and analyzed. All tests were conducted between 5 months and 3 years post-operation.

Results: Patients who underwent ACDF using vertebral spacer scored mean 2.22 in VAS and mean 6.66 in NDI whereas control group patient 4.66 and 16.22 respectively. Degenerative changes in analyzed group were less distinct than in control, nonetheless were visible in 6 cases. Bone fusion and thus vertebral stability observed in CT, scyntygraphy and SPECT was greater in patients with vertebral spacer than in control.

Conclusion: Diagnostic imaging studies correspond with clinical evaluation of patients using VAS and NDI scales. Overall ACDF with cage system seems to be a superior method having a lower risk of long-term complications and greater bone fusion.

Acknowledgements: Dr Jaroslaw Andrychowski for Supervising this project.

ORTHOPAEDICS

Healthy Limb Amputation: Ethical and Legal Issues

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Introduction: The presentation refers to the ethical and legal issues raised by patients who request to have a healthy limb amputated.

Material and Methods: Given all the possible and suggested origins of the desire for a healthy limb amputation, we present all the for- and against- thesis. Based on the most recent bibliography, court convictions, interviews with amputees and ethical parameteres, we attempt to approach the core of the permissibility or the prohibition of the requested surgery.

Results: Both sides-figthers and adherents of a healthy limb amputation support strongly their thesis. It is really important that each case should be examined in the context of uniqueness and thoughtfulness for the patients' and doctors' benefit and protection.

Conclusion: Given the ethically problematic history and doubtful effectiveness of such surgeries, surgeons should be very cautious before acceding to these requests. On the other hand, the establishment of a body of medical opinion on a healthy limb amputation is necessary for the patients' relief.

ORTHOPAEDICS

Diagnosis of Meniscal Tears and Anterior Cruciate Ligament Rupture: Clinical Examination Versus Magnetic Resonance Imaging

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Introduction: This study was performed to compare the clinical examination versus magnetic resonance imaging (MRI) in diagnosing meniscal and anterior cruciate ligament (ACL) pathology.

Materials & Methods: The study was conducted at Imam Hospital, which is one of the largest referral hospitals in Tehran, Iran. From April 2002 to April 2005, we evaluated 107 patients with an average age of 27.9 years suffering from 120 pathologies of the knee who were referred to our center. Each patient underwent a detailed and special physical examination by an experienced orthopedics surgeon. MRI examination was performed for the patients who had not been undergone MRI examination at other centers throughout the country. The senior author performed the arthroscopic procedures for all of our patients. Arthroscopy was considered to be the gold standard to evaluate the results of MRI and physical examination. Accuracy, sensitivity, specificity, and positive and negative predictive values were calculated comparing clinical examination, MRI, and arthroscopic evaluation.

Results: There were 61 ACL tears, 41 medial meniscus tears, and 18 lateral meniscus tears. The accuracy of clinical examination for ACL tearing ,medial meniscus tearing and lateral meniscus tearing were 90.5 (•CI=83.6-94), 74.3(•CI =65.4-81.1), 89.5(•CI =83.7-93) respectively. The accuracy of MRI examination for ACL tearing ,medial meniscus tearing and lateral meniscus tearing were 74.3(•CI=66-79.4), 54.3(•CI=46.1-60),79(•CI=72.9-85.2) respectively. The sensitivity of clinical examination for ACL tearing ,medial meniscus tearing and lateral meniscus tearing were 88.5(•CI=82.6-91.6),78(•CI=66.7-86.8),55.6(•CI=38.6-65.8) respectively. . The sensitivity of MRI examination for ACL tearing ,medial meniscus tearing and lateral meniscus tearing were 63.9(•CI=56.8-68.3),85.4((•CI=74.9-92.7),44.4(•CI=26.4-62.5) respectively. The specificity of clinical examination for examination for ACL tearing ,medial meniscus tearing and lateral meniscus tearing were 93.2(•CI=84.9-97.4),71.9(•CI=64.6-77.5),96.6(•CI=93-98.7) respectively. The specificity of MRI examination for ACL tearing ,medial meniscus tearing and lateral meniscus tearing were 88.6(•CI=78.7-94.7),34.4(•CI=27.7-39.1),86.2(•CI=82.5-89.9) respectively.

Conclusion: A well-trained qualified surgeon can safely rely on clinical examination for
diagnosing meniscal and ACL injuries. The routine ordering of an MRI scan of the knee before examination by a well-trained orthopedic surgeon is not recommended.

PEDIATRICS

Use of Video Capsule Endoscopy in Investigating Gastrointestinal Diseases in Children

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Introduction: Video Capsule Endoscopy (VCE), also called wireless capsule endoscopy, is an approved non invasive technique for assessing small bowel disease but data in children is limited. Currently the PillCam™ is licensed for use in children over the age of 10[1]. Indications for performing VCE include obscure GI bleeding, iron deficiency anemia, Crohn's disease, suspected small intestinal tumors, polyposis syndromes surveillance and suspected or refractory malabsorptive syndromes.

Material and Methods: This was a retrospective review to assess single centre experience of VCE in children over a 3 year period. The objective was to study the indications for VCE in children and assessment of its safety, tolerability and diagnostic yield compared to standard investigations in the pediatric population. Case notes and reports of 13 children, age range 11.7-17.5 years (median 15.1) from 2005-2008 were reviewed. VCE was performed with the Given Imaging ingestible capsule.

Results: In the 18 months preceding VCE,12/13 children had a colonoscopy and recent Barium follow through or abdominal MRI to exclude strictures. 8/13 children had pre existing conditions (lymphangiectasia n=2, Crohn's n=5, Indeterminate colitis n=1)
Indications
Abdominal Pain (n= 4), Rectal Bleeding (n=4), Assessing disease extent (n=4), diagnostic (n=1)
Safety and Tolerability
No Obstruction (n=13)
Failed to leave stomach (n=1)
Capsule placed endoscopically under general anesthetic (n=1)
Small Bowel Passage Time: 2h 19 m – 7h 15 m
Failure to reach caecum (delayed transit n=2, battery died n=1)
Diagnostic Yield
Lymphangiectasia: Both patients (n=2) had extensive disease therefore surgery not possible. Bleeding source: not found (n=4)
Crohn's disease: pathology mild so treatment not escalated (n=2)
Abdominal Pain: not IBD related (n=3)
Diagnostic: CD excluded (n=1)

Conclusion: VCE is a safe, well tolerated, useful investigative tool and its results can influence long term management in older children. However more research needs to be done to look at adapting VCE for use in younger children.

Acknowledgements: Dr Sandhia Naik for supervising this work.

PEDIATRICS

Vitamin D Receptor Gene Polymorphism and Bone Status in Children Treated for Cancer

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Introduction: Several reports show relation between calcium metabolism and vitamin D receptor (VDR) gene polymorphism. In particular calcium absorption and accretion seem to be genetically determined. Other environmental factors, as prolonged bed rest, frequent and long hospitalizations, steroids use in therapeutic protocols, may influence bone remodeling. Recessive homozygote of Fok I (ff) is postulated to be responsible for deficits in total body calcium accretion. This study aimed at analyzing a VDR genetical status in treated for cancer children and adolescents, who are at risk of poor bone metabolism.

Material and Methods: We have examined 176 patients with cancer (94 boys, mean age 14.82 ± 5.83) treated at the Department of Pediatric Oncology and Hematology of Medical University of Bialystok in Poland. Among those 64 subjects had leukemia, 41 lymphoma and 45 were diagnosed with solid tumors. Control group consisted of 26 healthy children (mean age 14.84 ± 5.32) from north-eastern region of Poland. The VDR Fok I gene polymorphism was evaluated by RFLP (restricted fragment length polymorphism) method, using polymerase chain reaction (PCR). Fracture status and calcium substitution dose were established for each patient by the leading physician.

Results: No significant differences in distribution of VDR genotypes, age, gender, calcium intake nor correlation with fracture status were found between patients and controls. In the studied population, higher prevalence of Ff genotype (52.8%) of VDR gene was found, which is consistent with data for Caucasian population. Analysis of the cancer group showed that in patients with leukemia the FF genotype was significantly more frequent (42,2%) than in solid tumors group (22,2%; $p<0,024$). Fractures occurred more frequently in the leukemia group (31,25%) than in the solid tumors (20%) and lymphomas (17,78%), although statistical significance between these groups wasn't reached. Among leukemia subjects with at least one f allele (ff and Ff) the tendency to greater number of fractures was observed in comparison to those without polymorphism ($p<0,053$).

Conclusion: 1. Fok I gene polymorphism established in this study is representative for Caucasian population. 2. In spite of protective genotype, assessed data suggests that children treated for leukemia are at higher risk of fractures than children with solid tumors, which may be explained by steroid therapy, longer treatment and not sufficient calcium bone accretion. 3. Further analysis including association of the FokI gene polymorphism with bone mineral density assessed by dual energy X-ray absorptiometry (DEXA) and fractures is being conducted.

PEDIATRICS

Screening for Problems in Eyes of Children Study (SPECS): Can we Detect Refractive Errors without Cycloplegic Eye Drops?

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Introduction: Amblyopia is the most frequent visual disorder in children and can lead to permanent visual impairment. Significant refractive errors and strabismus are important amblyogenic risk factors and occur in 5-10% of children. The Plusoptix S08 and the Retinomax K-Plus-2 are commercially available non-cycloplegic autorefractors for measuring the refractive status in children. They are quicker, less invasive and more cost-effective than the current golden standard, cycloplegic retinoscopy. Moreover, they could be useful as screening tools.

Material and Methods: The Retinomax K-Plus-2, the Plusoptix S08 and cycloplegic retinoscopy were compared. 200 children visiting the Ophthalmology Outpatients Department were included (age range 3 months to 11 years). Measurements with the non-cycloplegic devices were made by one lay screener and cycloplegic retinoscopy was performed by five orthoptists. The findings were analyzed by assessing the agreement with Bland-Altman plots. Sensitivity and specificity for detecting amblyogenic risk factors with the Plusoptix S08 were determined, according to criteria of the American Academy of Pediatric Ophthalmology and Strabismus (AAPOS). Furthermore, we evaluated how the Plusoptix performed in detecting strabismus.

Results: There was a minus overcorrection for all methods compared to cycloplegic retinoscopy. Mean differences (95% limits of agreement) between Retinomax K-Plus-2 without cycloplegia and cycloplegic retinoscopy were –2.11 D (-5.33 to 1.10) and –0.06 D (-0.98 to 0.86) for the spherical equivalent and the cylinder, respectively. Mean differences between the Plusoptix S08 and cycloplegic retinoscopy for the spherical equivalent and the cylinder were –1.13 D (-3.58 to 1.32) and –0.23 D (-1.28 to 0.81), respectively. Retinomax K-Plus-2 with cycloplegia performed better with mean differences of –0.08 D (-1.23 to 1.06) and 0.03 D (-0.72 to 0.78) for the spherical equivalent and the cylinder, respectively.

Sensitivity and specificity for detecting AAPOS amblyopia risk factors were moderate to high for myopia, astigmatism and anisometropia but low for hypermetropia (39,2%) due to minus overcorrection. By adjusting the referral criteria of the Plusoptix S08, sensitivity was 73% but specificity declined. The Plusoptix S08 was able to measure refractive status in children with manifest strabismus up to 15º.

Conclusion: Due to poor agreement with the current golden standard of cycloplegic retinoscopy, the Retinomax K-Plus-2 without cycloplegia and the Plusoptix S08 are not useful in the practice of the ophthalmologist. The Plusoptix S08 may be useful as a screening tool if the problem due to minus overcorrection can be overcome.

PEDIATRICS

Congenital Cytomegalovirus in Children with Permanent Childhood Hearing Impairment: Hearing Screening and Development

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Introduction: Congenital Cytomegalovirus (CMV) is the most important non-genetic cause of hearing loss in children. The virus can be transmitted from mother to fetus through the placenta anytime during gestation. Only approximately 10 to 15% of children born with a congenital CMV infection are symptomatic at birth. Both symptomatic and asymptomatic children with a congenital CMV infection can develop sequelae during the first years of life. The most common sequelae are permanent childhood hearing impairment (PCHI) and general developmental delay. Congenital CMV may be diagnosed later in life, when symptoms suggestive of congenital CMV develop. CMV DNA can be detected in blood stored as dried blood spots (DBSs). This is the blood which is taken for metabolic and other screening during the first week of life and which is stored for 5 years.In the Netherlands there is a unique opportunity to compare the efficacy of detecting PCHI in children by distraction hearing screening (at 9 months) or by the newborn hearing screening (within 2 weeks of birth). Late-onset or progressive PCHI caused by congenital CMV might result in a normal result at newborn hearing screening within two weeks of birth. General development and congenital CMV-DNA detection are primary outcome measures.

Material and Methods: The DECIBEL-study population consists of children with PCHI (hearing loss of 40dB in the better ear) born in the Netherlands between January 2003 and December 2005, who were offered a Dutch hearing screening strategy during the first year of life. Medical records and parental questionnaires were used to evaluate the development of the children.

Results: To date 183 children with PCHI participate in the special themes of the DECIBEL-study. Preliminary results of CMV-DNA testing show that approximately 8 % of these children have a congenital CMV infection. In the neonatal hearing screening strategy a greater number of children with PCHI with a congenital CMV-infection passed the initial hearing screening. Developmental outcome is significantly delayed in congenital CMV-positive children with PCHI compared to congenital CMV-negative children with PCHI.

Conclusion: The prevalence of congenital CMV in children with PCHI in the Netherlands is lower than reported in the international literature. Some children with a congenital CMV infection pass the neonatal hearing screening but have PCHI at a later age presumably because of progressive and delayed onset hearing loss. PCHI caused by a congenital CMV infection is regularly accompanied by general developmental delay.

PEDIATRICS

DECIBEL-study: Emotion Comprehension in Children with Permanent Childhood Hearing Impairment

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Introduction: In the Netherlands there is a unique opportunity to compare the efficacy and developmental consequences of detecting Permanent Childhood Hearing Impairment (PCHI) in children by the distraction hearing screening (at 9 months) or by the newborn hearing screening (within 2 weeks of birth). Newborn hearing screening is expected to lead to early intervention which suggests improved outcome in speech and language development, with as secondary effect improved chances for better socio-emotional development. In children with PCHI socio-emotional development is expected to be impaired. Not much is known about socio-emotional development in children with moderate to severe hearing-impairments who had access to early intervention. The objective of this study is to examine one key aspect of children's socio-emotional development - emotion comprehension - in children with PCHI..

Material and Methods: 195 children with PCHI participate in the DECIBEL-study. During home-visits, 59 children aged 3 - 5 years old with a bilateral hearing loss of at least 40 dB and fitted with hearing aids were seen. 36 children received the newborn hearing screening strategy, 23 children received the distraction hearing screening strategy. These children used and understood spoken language, made sentences of at least two words and did not use or need sign-language in communication with others. Emotion-comprehension was investigated by emotion-evoking situation tasks.

Results: Children in the distraction hearing screening strategy had a higher mean chronological age (54,7 months; 64,0 months); and higher age at hearing-aid fitting. No difference in emotion comprehension-scores was found between the two groups.

Conclusion: Emotion comprehension-scores did not differ between the two groups. However, the chronological age of the children was significantly higher in the distraction hearing screening strategy. This age difference suggests that emotion comprehension in children with PCHI is better following newborn hearing screening and early intervention than following distraction hearing screening and later intervention. Better emotion comprehension will improve the quality of children's social interaction with peers and will support meaningful social participation in life.

PEDIATRICS

The Effect of Music Instruction on the Cognitive Abilities of Preschool Children

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Introduction: Music instruction is known to enhance the spatial-temporal abilities in children. The aim of the study was determining the effects of short-term music instruction on general reasoning abilities and its four main components in preschool children.

Material and Methods: Thirty students of music classes at the age of 5 to 6 years at the beginning of the course of music were assigned to the experimental group. Another thirty kindergarten children with no music instruction were enrolled as the control group and the maternal education, economic level of the family, age and gender of the children were matched in two groups. The experimental group participated in a 12-week, vocal and instrumental music curriculum. The cognitive test used in this study was Tehran-Stanford-Binnet intelligence scale used as pre- and post-test for each sample.

Results: Statistical analysis showed significant gains for participants receiving music instruction in general reasoning ability. Moreover, they scored significantly higher than the "no music" group on the verbal reasoning and short-term memory subtests. The numerical and visual/abstract reasoning abilities did not differ for the two groups at the end of the course.

Conclusion: This study indicate that music instruction is useful to enhance some aspects of cognitive abilities e.g., general reasoning, verbal reasoning and short-term memory in preschool children.

PEDIATRICS

Non-immune Hydrops Fetalis in the LUMC Between 1997 and 2007: Diagnosis, Treatment and Outcome

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Introduction: Hydrops fetalis, a clinical picture in which the fetus develops edema and/or retains fluid in body cavities, occurs in 1 in 3000 pregnancies. Nowadays, 85 – 90% of these cases have non-immune hydrops fetalis, meaning maternal antibodies against fetal erythrocytes do not explain the etiology of the hydrops. Mortality rates in non-immune hydrops fetalis (NIHF) are reported between 60 and 90 %.

This research was done to get an overview of NIHF observed in the LUMC in the last 10 years. All cases were analyzed to acquire more information about this disorder, to improve diagnostic protocol and to eventually find new ways to reduce morbidity and mortality.

Material and Methods: A database was created of all the NIHF cases diagnosed prenatal from 1997 till 2007 in the obstetric department in the LUMC. Frequencies of causes, diagnostic methods, severity, treatment and outcome were analyzed. A multinominal regression test was used to see whether the treatment, severity of hydrops and diagnosis have an independent influence on the outcome.

Results: From the 161 cases of non-immune hydrops fetalis 40% had an unknown cause, 29% of all cases had an anemic cause and 31% had a non-anemic cause. Most common known causes of NIHF were parvo-infections (23%) and twin-to-twin transfusion syndromes (8.7%). Of the study population 73% received treatment. Most applied treatments were intrauterine transfusion (44%) and placement of a shunt (16%). Overall mortality was 47%. The survival percentage in the treated group was 59%, in the untreated group the percentage survivors is 36%. The multinominal regression test showed that the parameters diagnosis, severity of hydrops and treatment influence the outcome independently. From the cases with a neonatal follow up, 71% was correctly diagnosed prenatal.

Conclusion: The single most prevalent cause of NIHF in our department was anemia caused by parvo-infection. Diagnosis of anemia and/or of this infection should therefore be the first to be ruled out. In the diagnostic methods PCR showed superior to serological tests when infection was suspected. Negative serological tests should therefore be confirmed by PCR tests.

In our study population more than half of all NIHF cases survived, in contrast to most reports in the literature, even in cases where etiology remained unknown, or in severely hydropic cases. Treatment improved the outcome, both in the group of known and unknown causes. Symptomatical treatment should therefore be considered even when a final diagnosis is not yet achieved.

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PATHOLOGY

Kuppfer Cell Density and Distribution in the Livers of Patients with NAFLD, NASH and Normal Controls

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Introduction: Non-Alcoholic Fatty Liver Disease (NAFLD) is fatty inflammation of the liver in the absence of excessive alcohol use. It is related to the metabolic syndrome. NASH (Non-Alcoholic Steatohepatitis) is progressive form of NAFLD, is distinguishable from simple steatosis and characterized by necro-inflammation of the liver with fatty degeneration of hepatocytes. NASH can progress to liver fibrosis and cirrhosis and eventually to the development of hepatocellular carcinoma, has become common in the world. Ethiopathogenesis of NASH is not fully understood. Cytokine release from inflammatory cells (Kuppfer cells) is among the factors thought to be important in the pathogenesis. Kuppfer cells (Liver Macrophages) provoke inflammation by producing cytokines.

The aim of this study was to detect Kuppfer cell density and distribution in NAFLD, NASH cases and controls and try to determine whether there is a relationship between the morphologic appearance and topographic localization of the Kuppfer cells.

Material and Methods: To fully evaluate the role of Kuppfer cells in NASH, 89 needle biopsies showing NAFLD, NASH were selected for immunohistochemical staining to analyze the quantity and distribution of all Kuppfer cells. We also used 17 normal liver biopsies as a control. CD68 antibody was used as a marker of Kuppfer cells. Cases were selected by clinical and histopathological properties and classified with Brunt criteria's.

Results: In this study, we for the first time, demonstrate that although there was no difference in the quantity of the Kuppfer cells in the NASH and normal liver, the distribution of these cells was statistically significantly different in the NASH patients when compared with controls (p=0,0001). The aggregate formation by Kuppfer cells was quite noticeable. Aggregates were 68,25% in NASH , and 0.0% in the normal liver. Similarly, lipogranulomas were predominantly present in the NASH group (60,32%), and 0.0% in the normal livers (p=0,0001). The Kuppfer cells were localized lobularly in the normal livers, however in the NASH patients, 41,2% of them were observed in portal area in addition to lobular (p=0,015).

Conclusion: Inflammation in liver is a major component of the pathogenesis of NASH and progression to fibrosis (cirrhosis). It appears that Kuppfer cells are very important in inflammation. The cytokines that interact with inflammatory cells may be released by the Kuppfer cells of the liver. These cells should be investigated rigorously in order to understand pathogenesis of NASH further.

PATHOLOGY

Progressive Muscular Dystrophies: Constantine University Hospital

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Introduction: - The progressive muscular dystrophies (PMD) represent a group of genetically determined hereditary illnesses bound to a primitive degeneration of the muscular fiber.

- The PMD affects muscles which have reached their structural maturity and which are the siege of a necrosis followed by a regeneration of the muscular fiber with a fat infiltration.

Material and Methods The main progressive muscular dystrophies:
A. The muscular dystrophies bound to the dystrophin
- They constitute the first cause of myopathy for the child.
The dystrophin: Protein of 427kda, represent 0,002 % of total proteins of the muscle. It is situated in the internal face of the membrane connected with the extracellular matrix by the DAG (Dystrophin associated glycoprotein). The gene which codes for the synthesis of this protein is located on the short arm of the X chromosome in (Xp21).
- The dystrophin is abnormal, with a decreased molecular weight: Muscular dystrophy of Becker, or it can be absent: Muscular dystrophy of Duchenne de Boulogne.
1. Muscular dystrophy of Duchenne de Boulogne:
- It is the most frequent and the most severe PMD.
- Begin at the age of 3-4years; the first signs arise during the acquisition of walking, with a driving deficit of belts, pseudo hypertrophic calves and tendinous shrinkage. - Associated signs: cardiac infringement, mental delay.
-The muscular enzymes (CPK-LDH) are raised.
B. Muscular dystrophy of Steinert
- It is the most frequent PMD of the adult. - Associated signs: cardiac, endocrine, and eye infringements. - Age of the beginning: young Adult (20-30years).
D. The disease of Landouzy Déjerine E. Limb Girdle Muscular Dystrophy.

Results: - The PMD forms a very heterogeneous group of hereditary pathologies.
Three fundamental data are necessary for their classification:
1. The age of the beginning of clinical symptoms.
2. The distribution of the driving deficit.
3. The genetic study.
- They have for common denominator the dystrophic aspect put well in evidence in histological examination of a taking of muscular tissue (biopsy).

Conclusion: Big efforts were put together in order to try to develop therapeutic strategies, it is about the genetic and cellular therapy unavailable at the moment to us in Africa.

PATHOLOGY

Morphological Characteristics of RenalTransplant Needle Biopsies in University Clinical Center of Tuzla from 2000 to 2008

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Introduction: Renal transplantation represents the only possible therapeutic intervention for a large number of end-stage renal diseases. Living-related renal transplantation at University Clinical Center Tuzla was first implemented in year 1999, and first deceased-donor kidney transplantation was carried out in 2006. Transplant reactions occur when immune system of the recipient of a transplant attacks the transplanted organ or tissue, and they can be hyperacute, acute, and chronic. Renal allograft biopsy is essential to confirm the diagnosis of allograft rejection. Aim of this study was to determinate distribution and type of morphological changes, frequency of acute and chronic transplant rejections among renal biopsies performed at University Clinical Center Tuzla over an eight year period.

Materials & Methods: Needle biopsy findings from Policlinic for Laboratory Diagnostics - Department of Pathology, University Clinical Center Tuzla from 2000 - 2008 period were analyzed. Tissue samples were treated according to standard renal transplant protocol. They were analyzed by light-microscope, and detected morphological changes were classified by revisited BANFF classification. The study was retrospective.

Results: From January of 2000 to October of 2008, findings of total 269 renal biopsies were analyzed, out of which 59 were needle biopsies of renal transplants. Acute/active rejection with 30,49%; 37,3% of chronic/ sclerosing allograft nephropathy, as well as 10,2% of chronic rejection and 5,08% of chronic cyclosporine nephrotoxicity were found among analyzed needle biopsies of renal transplant. Thus, some changes that do not correlate with rejection were found, such as glomerulonephritises with 10,2% presentment. Normal findings made 1,7%, and 6,7% of findings were not interpretative..

Conclusion: Results of our study correspond with newest world reports, in which there is a noticeable tendency of decrease of frequency of acute rejection in kidney transplants, with increased frequency of chronic rejection reactions, interpreted with more adequate immunosuppressive therapy.

Analyzing renal transplant needle biopsies is crucial to determination of graft status in transplanted patients, and it serves a clinician as a guideline for future treatment of a patient.

PATHOLOGY

The Immunohistochemical Expression of E-Cadherin in Gastric Cancer. Correlations with Clinicopathalogical Factors and Patients´ Survival.

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Introduction: The aim of our study is to investigate the immunohistochemical expression of E-cadherin in gastric carcinomas and in surrounding mucosa (normal or with lesions of chronic atrophic gastritis, intestinal metaplasia or dysplasia).

Material and Methods: We included 61 patients with gastric cancers operated in Clinical Emergency County Hospital Timisoara, Romania. We analyzed the E-cadherin immunohistochemical expression, the correlation with clinical and pathological factors and outcome of the patients. The positive homogeneous pattern of staining for the cellular membranes is considered normal. Aberrant E-cadherin expression was represented by the negative homogeneous or the heterogeneous pattern (of the cytoplasm and membrane).

Results: Areas of chronic atrophic gastritis and intestinal metaplasia presented a normal pattern of immunostaining for the membranes. Aberrant E-cadherin expression was noticed in 30 cases of gastric carcinomas (49.2%) and in 11 cases (35.5%) of epithelial dysplasia in the surrounding tissue. Our results showed no correlation between E-cadherin expression and gender, age, tumor location, pT, pN, pTNM and lympho-vascular invasion ($p>0.05\%$). Aberrant E-cadherin immunostainings were significantly more frequent in diffuse-type carcinomas in comparison with intestinal-type carcinomas (82.4% vs. 31.6%) ($p=0.000491$ ES). Signet-ring carcinomas and anaplastic carcinomas presented a high proportion of aberrant immunostainings (82.4% and 100%), as well as poor differentiated carcinomas (61.5%). Carcinomas with distant metastasis presented significantly more aberrant immunostainings than those without metastasis (71.4% vs. 42.6%) ($p=0.03246$ S). Five years survival rate was significantly lower in patients with aberrant E-cadherin expression in comparison with the patients presenting normal staining (10% vs. 22.6%) ($p=0.02693$ S).

Conclusion: In gastric carcinomas and areas of epithelial dysplasia, aberrant E-cadherin expression was significantly more frequent in comparison with the surrounding normal mucosa ($p< 0.001$ ES). Our data suggest a strong correlation between Lauren classification of gastric carcinomas and E-cadherin immunohistochemical expression. Assessment of the survival curve of the patients highlighted the role of prognostic factor for the aberrant immunohistochemical E-cadherin expression.

PATHOLOGY

Mitotic Activity and a Diameter of Tumor as Prediction Factors of Aggressiveness in Gastrointestinal Stromal Tumor

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Introduction: Gastrointestinal Stromal Tumor (GIST) is the most common type of a rare group of neoplasms, which are mesenchymal tumors. GISTs can appear in any part of the gastrointestinal tract but the most common localization is stomach and small intestine. Nowadays the interstitial cells of Cajal (ICC) are a potential origin of the neoplasm cells. As a consequence in GIST cells, there are some characteristic for ICC proteins, which are expressed (e.g. type 3 tyrosine kinase c-KIT), what has an essential meaning for the diagnostic criteria. Although GISTs are generally malignant neoplasms, the degree of aggressiveness is variable and depends on size of tumor, mitotic count and localization as well. Therefore the aim of the study was to examine the mutual relationship between size, aggressiveness degree and mitotic count of GIST, and to analyze the background of the correlations.

Material and Methods: The study was based on medical records of 15 patients who underwent an operation for tumor obliteration in the Department of General, Oncological and Thoracic Surgery (Central Clinical Hospital Military Institute of Medicine in Warsaw) between 2002 and 2008. The correlation of appropriate parameters was performed using the statistical analysis methods (Pearson's correlation).

Results: The correlation between mitotic activity (expressed by the amount of mitosis per 50 high-powered field, HPF) and the highest diameter of the tumor (in centimeters, cm) expressed by Pearson's correlation coefficient was -0,18770, what can suggest, that there is no relationship between the characteristics. However we found two correlations: the first one is the correlation coefficient of the relationship between the mitotic activity and the aggressiveness degree of the tumor, which is 0,69075. The second correlation was found between the highest diameter and the aggressiveness degree of the tumor, and it was 0,576419.

Conclusion: Absence of the correlation between mitotic activity and the highest diameter of the tumor as well as presence of the correlation between mitotic activity and the aggressiveness degree and the highest diameter and the aggressiveness degree are consistent with previous studies. The correlations show, that if the mitotic activity and the diameter of the tumor increase, the aggressiveness of the tumor also rises, what can predict the outcomes of the patient and the appropriate methods of medical treatment.

PATHOLOGY

Morphological and Biochemical Aspects in Cerebral Hypoxia

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Introduction: Being a damaging process for the nervous tissue, especially for the cerebral part of CNS, hypoxia has numerous effects on the function and structure of neurons. In this study are described macroscopic and microscopic changes of the rat brain, precisely cortex and cerebellum, and biochemical serum analysis in search of certain enzymes, ions and pH data concluding the changes. All these regarded the comparative evaluation of hypoxic modifications as a result global incomplete ischemia versus that as a result of global complete ischemia, at the end sustaining the idea the first mentioned type is more aggressive.

Material and Methods: The experimental study was made on 15 Wistar rats (10m, 5f), 8 months age, and weight 170-195 g ±4. They were divided into 3 groups, 2 experimental lots (I and II), each of 6 rats, and one for control (III). To subjects in the first group was applied common carotid artery bilateral ligature and for those in the second – induced hemorrhagic shock, by extracting from the external jugular vein 3.5ml ± 0.4 of blood, in both cases the exposure time lasting 5 minutes. Serum analysis had interest for Ca2+ ions, lactic acid and alkaline phosphatase (ALP) also the histological and morphological observations were done.

Results: Macroscopic signs revealed were edema of the white matter in the hemispheres, in hippocampus, corpus callosum, cerebellum and per diapedesis hemorrhage. Histological observations revealed pericellular and intracellular edema, extravasation blood particles nearby vessels, neuron nucleus hypercromativity, irregular neuron and glial cells form, vessels' rupture, Purkinje cells' alteration. All these signs were better illustrated in the subjects of the first experimental lot.
Biochemical analysis showed: a) Ca2+ (C% mM/l): I-3.35±0.21, II-4.06±0.20, III-5.27±0.21; b) Lactic acid (C% mM/l): I-6.2±0.39, II-5.6±0.34, III-4.96±0.44; c) ALP (A Nm/s*l): I-0.63±0.12, II-0.53±0.12, III-0.39±0.09.

Conclusion: This study demonstrated that global incomplete ischemia is more damaging than the complete form, considering the morphological and biochemical data obtained. In such way the hemorrhagic shock form is less destructive for the CNS, especially it's cerebral part.

PHARMACOLOGY

Essential Medicines List for Children: First Report on Proposed Essential Medicines for Neonates

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Introduction: Despite the technical advances that have been achieved, many gaps related to neonatal health care remain globally. The substantial lack of evidence regarding the efficacy and safety of many medicines used in the treatment of neonates and the scarcity of appropriate formulations for this age group was recognized during the First Meeting of the World Health Organization (WHO) Subcommittee of the Expert Committee on the Selection and Use of Essential Medicines in July 2007. Therefore, a review was conducted to identify potential essential medicines for neonates to be considered for inclusion in the Essential Medicines List for Children (EMLc). This list will ultimately improve suboptimal prescribing and dosing of medicines in this vulnerable age group by guiding national formularies and contribute to a public health oriented research agenda by identifying research gaps.

Material and Methods: The objectives of this study were (1) To identify clinical problems treated in primary, secondary and tertiary level of neonatal health care. Clinical problems were derived from National Guidelines Clearinghouse, the Canadian Pediatric Society, Neonatal Handbook-Victoria (Australia), and the British Association of Perinatal Medicine. (2) To identify medicines currently recommended in WHO and other guidelines for use for each of these clinical problems; (3) To identify clinical evidence to support the use of these medicines. A wide spectrum of sources were searched, including Cochrane Reviews, BNF for Children, The Neonatal Formulary, E-medicine, Database of Abstracts of Reviews of Effects, International Child Health Collaboration, Centers for Disease Control and Prevention, and RxList. (4) To identify prices of each of the selected medicines in the International Drug Price Indicator Guide or British National Formulary for Children 2006 to determine cost-effectiveness. Also, the licensing status of selected medicines was checked.

Results: As expected, data on many medicines were limited. In addition, a substantial proportion of recommended medicines in guidelines do not have supporting evidence or have more effective or safer alternatives. However, several medicines were identified as potential essential medicines for neonates. Expert judgements were exercised during meetings of the Subcommittee in October 2007 and September 2008. On the basis of the review, medicines currently missing from the EMLc, and recommended exclusively for use in neonates were identified, and included in the EMLc.

Conclusion: The paucity of high quality evidence for the use of medications in the neonatal period and the subsequent off-label and unlicensed use in this population are major problems. A more detailed and systematic review of available evidence for efficacy and safety of the medicines recommended for neonates may be required to ensure neonates receive optimal Medical care.

PHARMACOLOGY

The SAR Studies of Some α1-adrenocoptor Antagonist and Antiarrythmic Agents

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Introduction: The arylpiperazines represent one of the most studied class of α1-AR receptor antagonists (α1-AR). The typical α1-AR contains arylpiperazine moiety, substituted with one or two additional molecular fragments. The pharmacological results suggest that the antiarrhythmic effects of these compounds are related to their adrenolytic properties.

Material and Methods: The synthesis of a series of 1-[3-(4-arylpiperazin-1-yl)]propyl-pyrrolidin-2-one derivatives were previously reported[1]. Some of these compounds shows high affinity for α1-AR, and in in vivo research displayed antiarrhythmic activity. Two subsets of the most active compounds listed below were analysed by means of the Structure Activity Relationship (SAR):
A0 : 1-[3-(4-(2-etoxyphenyl)piperazin-1-yl)propyl]pyrrolidin-2-one
A1: 1-[3-(4-(2-etoxyphenyl)piperazin-1-yl)-2-hydroxypropyl]pyrrolidin-2-one
A2: 1-[3-(4-(2-etoxyenyl)piperazin-1-yl)-2-hydroxypropyl]3,3-diphenylpyrrolidin-2-one
B0 : 1-[3-(4-(4-metoxyphenyl)piperazin-1-yl)propyl]pyrrolidin-2-one
B1: 1-[3-(4-(4-metoxyhenyl)piperazin-1-yl)-2-hydroxypropyl]pyrrolidin-2-one
B2: 1-[3-(4-(4-metoxyenyl)piperazin-1-yl)-2-hydroxypropyl]3,3-diphenylpyrrolidin-2-one
In order to define the molecular structure of investigated compounds, a molecular modeling study was undertaken. The three-dimensional structures of the pyrrolidin-2-ones derivatives at their neutral state were obtained through full optimization based on the AM1 quantum chemical procedure. Force field calculations were used to ascertain whether the resulting structures were energy minima. All the molecules were minimized until the root mean square (RMS) gradient value became smaller than 10– 6 a.u. The single point energy calculations were performed at the DFT/B3LYP level of theory using the 6-31G** basis set. Our SAR study involve the charge distribution in the pharmacophore plane. Suitable maps of electrostatic potential was plotted due to electronic and nuclear charge distribution from the energy calculations results. The Gaussian suites of programs calculates the electrostatic potential maps and surfaces as the distribution of potential energy of a unit positive charge in certain molecular space with resolution controlled by the grid density.

Results: According to the very basic theory of electrostatics the negative potentials corresponds to attraction of the probe unit charge by the higher electron density in the space, while the positive potentials corresponds to repulsive interactions of probe charge with unshielded nuclear forces present in the low electron density spaces.

Conclusion: The results of this computer modeling study indicates that if the arylpiperazine terminal moiety is surrounded by the regions of negative electrostatic potential (resulting from presence of 4-metoxyphenyl substituent of piperazine moiety) then the antiarrythmic activity is blocked.

PHARMACOLOGY

Experimental Research of Analgesic Effects of PL3a and PL1 Fractions Obtained from *Plantago lanceolata*

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Introduction: The objective of the present study was to investigate the possible analgesic effect of the phenylethanoidic (46.57% acteoside) and phenylethanoidic-iridoidic (18.78% acteoside, 4.94% catalpol, 7.84% aucubin) fractions obtained from Plantago lanceolata. The compounds were quantified by HPLC and the absence of flavonoids and iridoids was confirmed by negative color reactions with specific reagents.

Material and Methods: The „writhing test" and the „hot plate test" were conducted on test groups of 8 albino mice injected intraperitoneally with 215 mg/kg of phenylethanoidic fraction or with 532 mg/kg of phenylethanoidic-iridoidic fraction thereby the acteoside dose being 100 mg/kg for all the test groups. The control groups were injected with saline solution 10 ml/kg. The analgesic effect of these two fractions was evaluated at different periods of time after solutions administration (15, 30, 60 and 120 minutes). The results were statistically analyzed with one-way ANOVA and Dunnett's test.

Results: The phenylethanoidic and phenylethanoidic-iridoidic fractions showed an analgesic effect in the "writhing test" at 30 and 60 minutes after administration ($p<0.05$) and the intensity of this analgesic effect was the same for both fractions as compared to the control groups. In the "hot-plate test" no difference between the first reaction latency of the test groups and control groups was observed.

Conclusion: The phenylethanoidic and the phenylethanoidic-iridoidic fraction had a slow-onset and short-duration analgesic effect demonstrated only in the "writhing test". The analgesic effect determined by the studied fractions could be due to a peripheral non-steroidal anti-inflammatory mechanism, but the active substance responsible for this effect has not been yet identified.

PHARMACOLOGY

Effect of Music on Pain Related to Propofol Injection

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Introduction: Propofol, a very common anesthetic drug, can induce pain at injection site in 70% of patients. Music can distract from the pain sensation. As Mozart's Sonata for Two Pianos in D Major, K448 induces the best quantified effects on cerebral activity, we aimed to investigate whether it could decrease pain sensation. The goal of our study was to investigate if Mozart's K448 Sonata modifies the pain sensation due to propofol injection.

Material and Methods: Adult patients (without chronic analgesic medication) undergoing digestive endoscopies in one hospital were included in this prospective, randomized, controlled study. They were randomly assigned either to K448 group or to control group. The K448 group patients listened to Mozart's K448 from their arrival in the operating room to the complete induction of general anesthesia. Control group patients had a similar anesthetic procedure, without any music. The patients received half of the propofol dose used to induce anesthesia (10mg/ml, 1mg/kg), and were then asked to quantify their injection related pain on a 1-to-10 numeric scale. Afterwards, sufentanil (10 μg), midazolam (1mg) and the rest of the induction propofol dose were injected. In the recovery room, 15 minutes after their awakening, the patients were questioned about post interventional pain, pain site, their recall regarding listening to music, propofol injection and injection related pain. They were asked to quantify these pains on a 100 mm visual-analogue scale. Perfusion site, caliber and number of attempts for inserting the venous catheter were also noted. Our primary end point was the percentage of patients reporting propofol related pain in the operating room. We plan to include 40 patients per group to detect a 50% decrease in patients reporting pain with alpha risk of 0.05 and sensibility of 0.80. Data were analyzed using Fisher's or non parametric tests.

Results: At time of submission, 61 patients were included (19 K448 and 42 control patients). Both groups had similar demographic data. In the interventional room, propofol related pain was reported by 47% of K448 patients and 33% of control patients (p=0.15). In the recovery room, 42% of K449 group and 47% of control group reported propofol related pain (p=0.37). In both groups, propofol related pain intensity was similar (p=0.49), ranging from 0 to 80mm.

Conclusion: Based on these preliminary results, Mozart's K448 does not seem to decrease the propofol injection pain in operating room or in recovery room.

PHARMACOLOGY

The Long Term Impact of Albendazole-Ivermectine Mass Treatment on the Prevalence of Hookworm and Strongyloides Infections in Northern Ghana

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Introduction: Soil transmitted helminths (STH), including among others *Strongyloides stercoralis* and the hookworm species *Necator americanus* and *Ancylostoma duodenale*, are major contributors of tropical diseases in developing countries. Estimates of infected people range from 700 million to 1.3 billion. Although mortality rates are low, these intestinal nematodes are considered to be an important public health problem and are responsible for high morbidity and many Disability Adjusted Life Years. Attempts to control soil transmitted helminths are aimed at mass treatment and improvement of environmental conditions. Currently vaccines are being developed.

Material and Methods: The Department of Parasitology of the Leiden University Medical Centre has a long history of epidemiological research on soil transmitted helminths in Northern Ghana, a region known for its high prevalence of the STH *Oesophagostomum bifurcum*, *S. stercoralis*, and *N. americanus*. The current study evaluates the impact of several rounds of mass treatment with albendazole combined with ivermectine on the distribution of the latter two species. In one particular region, named Garu area, three additional rounds of mass treatment with albendazole only were performed between October 2001 and October 2002.

Results: Now we collected 1417 stool samples from 26 randomly selected villages within and outside the Garu area and examined 1286 by coproculture procedure. A highly focal distribution was noted, but overall the prevalence of hookworm and *S.stercoralis* decreased substantially. Compared to the cross-sectional surveys performed before intervention took place in the Garu area, hookworm decreased from 86.9% to 18.6% and *S.stercoralis* from 16.5% to less than 1%. High intensity infections, meaning more than 100 hookworm L3 larvae in a single coproculture examination were virtually absent, while commonly seen before intervention took place. Similar findings were seen outside the Garu area.

Conclusion: This study indicates an important reduction, but no eradication in STH following mass treatment with albedazole and ivermectine. Our findings seem to be in contrast to previous surveys in the region predicting an almost complete recovery of pre-intervention hookworm prevalence within several years following mass treatment. Possible alternative explanations will be discussed.

PHARMACOLOGY

The Pharmacodynamics of ATG in Pediatric Patients

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Introduction: Stem cell transplantation (SCT) is a frequently used therapy for children with different malignant and non-malignant hematological diseases. Polyclonal anti-thymocyte globulin (ATG) is given prior to SCT to prevent rejection and Graft-versus-Host disease (GvHD).

Material and Methods: In this study the course of the ATG concentration was measured in a cohort of 18 patients who received their first SCT at the department of Pediatrics of the Leiden University Medical Centre (LUMC) between August ’06 and July ’07. Unique in this study was that serum samples were taken frequently before and after every ATG dose. It was also new that the concentration of active ATG was measured by FACS, whereas the total amount of rabbit IgG was measured by ELISA techniques.

Results: It was found that the ATG concentration showed peak and off-peak values between every ATG dose. There was a huge variation between the individual patients; the median active ATG concentration that was reached after the final dose was 12.0 U/ml with a range from 5.1 to 25.4 U/ml.

It was found that there is no good correlation between the amount of active ATG and total rabbit-IgG in a patient over the different time points pre and post SCT, therefore FACS is the most reliable method to measure the concentration of active ATG.

Although all patients received ATG in a dose of 10 mg/kg a significant difference (P=0.002) was found between the patients with a bodyweight below or above 30 kg, therefore there could be a risk of overdosing in this last group.

The clearance of active ATG is faster than the clearance of total rabbit-IgG with half-lifes of 2-7 days and 5-30 days respectively. In the first days after ATG administration the half-life of the active ATG is even faster.

Patients with a low ATG concentration at the moment of transplantation were found to have a higher risk to develop GvHD.
Conclusion: It would be ideal to develop a pharmacodynamical model for the dosing of ATG in individual patients, but further research with more patients is needed to make that possible.

PHARMACOLOGY

Chronic Administration of 5-HTT-Inhibitor Renders Dual Effect on the Development of MCT-Induced Pulmonary Hypertension in Rats

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Introduction: Pulmonary hypertension (PH) is characterized by right ventricular hypertrophy, vascular remodeling and high right ventricular systolic pressure (RVSP). There are suggestions that one of the pathogenesis factors of PH is the increase in plasma serotonin concentration. But the pathogenesis of PH is not fully understood and the treatment of PH is still undetermined. Objective: The purpose of this study was to investigate whether inhibition of 5-HT transporter by fluoxetine would prevent the development of monocrotaline (MCT)-induced PH in rats.

Material and Methods: Daily administration of fluoxetine (10 mg/kg/day per os for 3 weeks) or vehicle (placebo) was started 7 days after to a single-dose injection of MCT (60 mg/kg). On day 28, the change of RVSP, right ventricular hypertrophy, and medial wall thickness were assessed.

Results: RVSP (74,69±0,42 vs 33,50±0,70), medial wall thickness of pulmonary vessels (36,08±1,52 vs. 17,31±1,35) and right ventricular hypertrophy (36,37±1,29 vs. 20,98±0,47) were increased in rats with MCT-induced PH versus in control rats. Fluoxetine-treated rats divided into 2 subgroup by RVSP: in the first subgroup RVSP was significantly reduced (54,88±1,95 vs. 74,69±0,42), while in second subgroup RVSP was increased in comparison with rats which received vehicle (105,50±4,00 vs. 74,69±0,42). In rats with daily administration of fluoxetine right ventricular hypertrophy was reduced in the first subgroup (30,99±1,00 vs. 36,37±1,29) and remained unchanged in the second subgroup in comparison with rats with MCT-induced PH. In the first subgroup chronically treated with fluoxetine other effects of the inhibitor were also evident, such as loss of body weight (300±5,0 vs. 320±4,5) and an anorexic effect (23,5±0,42 vs. 26,6±0,62) versus in rats with MCT-induced PH. Medial wall thickness (24,03±1,35 vs. 36,08±1,52) of pulmonary vessels of fluoxetine-treated rats was less severe than in rats with MCT-induced PH.

Conclusion: We conclude that daily supplementation of fluoxetine renders a dual effect on the development of MCT-induced PH in rats. The results suggest that chronic administration of fluoxetine can lead to both reduction of the symptoms of PH and to progression of the illness.

PHARMACOLOGY

Etiology & Treatment Results of Bisphosphonate Related Osteonecrosis of the Jaws

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Introduction: Bisphosphonates are frequently used worldwide. There are several indications to prescribe bisphosphonates. The most important are osteoporosis and skeletal bone metastases in malignancies. Bisphosphonates decrease the function of osteoclasts and hence bone resorption. Bisphosphonates also have side-effects; one of the more severe being bisphosphonate-related osteonecrosis of the jaws (BRONJ). This can be difficult to treat and may lead to serious loss of parts of the jaw. However surgical treatment has also shown to lead to loss of the jaw. Therefore the literature recommends caution with surgery.

The exact pathogenenesis of BRONJ is unknown. In the literature trauma, dental pathology or spontaneous development is suggested. In this study 28 patients were analyzed retrospectively to determine pathogenesis and optimal treatment.

Material and Methods: 28 patients with exposed bone were seen in the Department of Oral and Maxillofacial Surgery of the Leiden University Medical Center. Of all patients clinical features, bisphosphonate use, dental history including luxating moment and (previous) treatment were studied.

Results: In 96,4% (n=27) a dental focus such as a previous dental procedure or denture complaints could be identified. 23 cases were treated with thorough surgery in combination with antibiotic therapy. 78,3% (n=18) healed and remained free of symptoms during follow-up varying from 6 to 99 months with an average of 23,0 months.

Conclusion: In contrast to findings in the literature in our group of patients a dental focus could be found in 27 of 28 cases. This may have consequences in the prevention and treatment of BRONJ. Treatment consisting of combined thorough surgery and antimicrobial therapy shows promising results.

PHARMACOLOGY

Protective Effects of *Nigella sativa* (black seed) Against Doxorubicin Dose Dependent Cardiotoxicity

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Introduction: Doxorubicin, DXR (*Andriamycin*) belong to the family of *Anthracyclines*, widely used for the treatment of Sarcomas and variety of Carcinomas, including breast and lung cancers. It is also employed in the treatment of Lymphomas and acute Lymphocytic Leukemia. DXR therapeutic use, however, is limited by its adverse, sometimes irreversible dose-dependent cardiotoxicity and bone marrow suppression, triggered through release of semiquinone free radicals, which *steals* electrons from molecular O_2, producing superoxide ions and hydrogen peroxide that mediate single strand scission of the DNA. Cardiac cells and tumors are low in ample superoxide dismutase, SOD, or glutathione peroxidase that mops up reactive oxygen species, ROS, making the former vulnerable to toxicity. Many compounds like garlic, mistletoe, *et cetra* believed to possess anti-oxidant properties have been studied for their potential protective agents against DXR induced cardiotoxicity. Black Seed, BSD, an annual herbaceous plant, native to the Mediterranean but cultivated also in the Middle East and Africa have been noted for its anti-tumor, antioxidants, antibiotic, hepatoprotective, anti-inflammatory and immune strengthening properties. These documented antioxidant properties, would it prove a useful therapeutic adjuvant against DXR treatment-generated free radicals induced myocardial toxicity?

Material and Methods: Albino rats were obtained and divided into 4-groups (n=5): the DXR treated group was acclimatized to oral daily dose of 5ml/kg/b.wt distilled water for 2wks, followed by single dose intraperitoneal injection of 10mg/kg/b.wt DXR after 7dys. The 2nd group was fed BSD liquid extract of 500mg/5ml liquid extract for 2wks, then given the same single dose injection of DXR after 7dys, preceding BSD treatment. The 3rd group received BSD extract for 2wks, then injected with one dose of 5ml/kg/b.wt after 7dys of BSD treatment. The control group received 5ml/kg/b.wt distilled water for 14 days, and then injected single dose saline after 7dys of water *treatment*. In the end, blood and heart tissues were collected from all the groups for further procedures.

Results: Serum marker of heart damage, AST, significantly increased ($p<0.001$) in DXR treated group compared to control. Group administered DXR with BSD showed slight increase in AST. Histological studies revealed several histopathlogical lesions associated with DXR treated group but moderate to normal preserved myocardial cells in DXR-BSD treated group.

Conclusion: Cardiotoxicity induced by DXR administration may be ameliorated with co-administration with BSD through acting as a sort of Interferon, with an immune booster and antioxidant efficacy.

PHARMACOLOGY

Simvastatin Decreases Hepatic Ischaemia/Reperfusion-Induced Liver and Lung Injury in Rats

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Introduction: Liver failure is still a significant clinical problem after transplantation surgery, tissue resections (the Pringle manoeuvre) and haemorrhagic shock. The restoration of blood flow to an ischaemic region leads to tissue injury at a greater rate than the original ischaemic insult, an event termed .ischaemia-reperfusion injury. (I/R). Despite advances in surgical techniques, I/R still poses a problem of clinical importance. In this research, we studied the effect of simvastatin pretreatment on liver and lung injury induced by hepatic I/R.

Material and Methods: Rats were subjected to 30 min of ischaemia followed by 24 h of reperfusion. Simvastatin (10 mg/kg) was administered orally from three days before the operation. After the reperfusion time, serum ALT, AST, LDH and TNFa levels were studied and liver and lung tissues were stained with haematoxylin and eosin and TUNEL to detect apoptotic cells.

Results: ALT(135 ± 14.1),AST(335 ± 17.1) and LDH(4558 ± 210) and TNFa (64.7 ± 5.2) levels were increased markedly by hepatic I/R, and these were suppressed significantly by simvastatin ALT(87 ± 13.3),AST(231 ± 15.4) and LDH(3317 ± 183) and TNFa (41.4 ± 4.1). The tissue injury index and the number of apoptotic cells via TUNEL staining in the liver and lungs were higher in the I/R group than in the I/R + simvastatin group significantly.

Conclusion: These results suggest that simvastatin ameliorates I/R-induced liver and lung tissue damage by inhibiting the level of inflammation and the apoptotic pathways. Simvastatin administration may therefore provide protection against the adverse effects of I/R injury in liver transplantation.

PSYCHIATRY

Emotional and Behavioral Problems among Street Children at Shelter Houses in Central Jakarta and Several Factors Associated

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Introduction: Negative environmental exposures faced by street children put them in a harmful condition of developing emotional and behavioral problems. Those problems may affect their life and provide bad consequences for society. Thus, identifying emotional and behavioral problems among street children and factors associated is needed to arrange effective prevention and interventional care. Objective of this study is to identify behavioral and emotional problems among street children in Central Jakarta and investigate several factors associated.

Material and Methods: This is a cross-sectional study. Samples were 88 street children aged 10-16, registered at shelter houses in Central Jakarta, and selected using consecutive sampling method. Data were collected using Strength and Difficulties Questionnaire (SDQ) and additional questionnaire. SDQ was used to identify behavioral and emotional problems; emotional symptom, conduct problems, peer problems, hyperactivity, total difficulties, and prosocial behavior.

Results: This study revealed that among the subjects, 15.3% had abnormal total difficulties score, 10.6 % had abnormal emotional problems score, 28,2% had abnormal conduct problems score, 11.8 % had abnormal peer problems score, and 1,2% had abnormal prosocial behavior score. None of subjects had abnormal hyperactivity problem score. History of sexual abuse was associated with total difficulties score, conduct problems, and peer problems; age had association with total difficulties and peer problems; addictive substances usage was associated with total difficulties and peer problems; whereas amount of days spent on street per week, length of time spent on street per day, and smoking were only associated with peer problems.

Conclusion: We found abnormalities in total emotional and behavioral problems, emotional problems, conduct problems, peer problems, and prosocial behavior problems. The associated factors found were age, history of sexual abuse, addictive substances usage, amount of days spent on street per week, amount of time spent on street per day, and cigarette smoking. Therefore, greater concern regarding to these problems, such as giving psycho-education and life skills education, is required to improve street children's quality of life and avoid further impacts.

PSYCHIATRY

Partial Animal Modeling of Schizophrenia

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Introduction: Strong evidence indicates that multiple, interconnected, cortico-limbic brain regions are involved in the pathophysiology of schizophrenia (SZ). Little is known about the relationships between abnormalities in each of these regions. These abnormalities may be primary, perhaps caused by genetic and/or environmental factors, or secondary, downstream effects of primary changes along cortico-limbic regions.

The aim of this presentation is to introduce a "partial" animal modeling strategy, which was developed to investigate cascades of secondary changes within interconnected brain regions. This strategy is designed to systematically dissect out, from a complex network of cortico-limbic connections, a discrete aspect of a specific neural circuitry.

Among the most consistent postmortem findings in the limbic lobe, including the hippocampus, anterior cingulate gyrus (ACG) and amygdala, are abnormalities of GABAergic transmission, involving specific interneuronal subpopulations. The model presented simulates a GABAergic deficit in a specific brain region, such as the amygdala or ACG, by local pharmacological blockade of GABA-A receptors. Neurochemical changes in the target regions of these areas are then investigated. Intraparenchymal local drug infusion can be performed chronically thus allowing to approximate the time course of the disease and can be adapted to different age ranges in order to test hypotheses on the onset of induced changes in target regions.

Material and Methods: Rats were used as the experimental animals. GABA-A receptor antagonist picrotoxin was stereotaxically infused into either the amygdala or ACG, where GABA defects are postulated to occur in SZ. Changes of numerical densities of specific interneurons in the hippocampus or amygdala respectively, were assessed, using immunocytochemical procedures. Survival time varied from acute to chronic and postnatal ages ranged from 25 to 60 days old.
Results: Picrotoxin infusion in the amygdala altered numerical densities of specific interneuronal populations in the hippocampus in a manner that closely reproduced abnormalities described in this region in SZ. Chronic picrotoxin infusion in the ACG was found to affect expression of GABA in the amygdala in an age-dependent manner.

Conclusion: These results support the construct and predictive validity of this model. Stepwise application of this rodent model to postmortem findings could help explain the links between neurochemical abnormalities in interconnected brain regions in SZ. Reciprocally, changes detected by this strategy in rodent models can be investigated in the corresponding regions in human tissue from SZs.

PUBLIC HEALTH

To study the Health Status of School Going Children: A Cross-sectional Study at Aliabad village in Andhra Pradesh India

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Introduction: In India, several studies have been carried out on the health status of school age children. These have largely been quantitative and the reported morbidity included malnutrition. There were very few comparative studies on morbidity status of school going children hence a cross sectional study was designed and carried out in July 2005-January 2006, to assess the nutritional status and morbid conditions among school going children in Government and Private schools of rural area.

Material and Methods: Using qualitative and quantitative research methods, a schedule for examination was designed. Children in age group of 6-10 years were examined and their observations were recorded as regards their sex. Age, height and weight were recorded to assess the nutrtional status according to the Gomez classification. A total of 409 children were examined (312 in Government schools and 97 in private schools).

Results: Around 82.37% children in Government schools and 58.76% of children in Private schools were found to be malnourished. It was also found that 57.19% of girls and 42.8% of boys in Government schools compared to 16.49% of girls and 42.26% of boys in Private schools were affected with malnutrition. Morbid conditions like Ear related ailments were found to be 31.73% in children from Government schools to 13.40% in children from private schools. About 12.17% children from Government schools suffered from skin related ailments compared to 8.24% in children from private schools.

Conclusion: There is a significant difference in nutritional status among school children from Government and Private schools in Rural areas as compared to other morbid conditions. Also observed that girls are more affected by malnutrition. It is of notice that girls are not equally cared on par with boys in rural areas especially in the lower socioeconomic groups.This shows improved socio economic status helps attain better health.

PUBLIC HEALTH

Emotional and Behavioral Problems and Their Association with Sexual Abuse among Street Children in Central Jakarta, Indonesia

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Introduction: The phenomenon of street children is a problem in Indonesia. Negative environmental exposures faced by street children put them in a harmful condition of developing emotional and behavioral problems. Those problems may affect their life and provide bad consequences for society. Thus, identifying emotional and behavioral problems among street children is needed to arrange effective prevention and interventional care. Furthermore, we searched association between one of the negative exposures, history of sexual abuse, and those problems.

Material and Methods: A cross-sectional study was done to a total of 88 street children aged between 10-16 years old at all 5 shelter houses in Central Jakarta. Questionnaires were used to collect data on characteristics and history of sexual abuse. Emotional and behavioral problems were assessed using Strength and Difficulties Questionnaire (SDQ), which analyzed emotional symptom, conduct problems, peer problems, hyperactivity, total difficulties, and prosocial behavior.

Results: The characteristic of street children were mostly male (68.2%), mean age of starting to be street children is 10,51 (SD 3.18), still attend school (65.9%), Both parents are still alive (83%), didn't exposed to parental divorce (87.5%), still have parental support (90.9%), being street children for more than 6 months (55.7%), spent 4-6 hours on street per day (68.2%), spent less than 3 days per week on the street (43,2%), have a job as street musician (45,5%), main reason to be street children is poverty (59,1%), and have been sexual abused (60.2%).

Emotional and behavioral problems found were emotional symptom (10.6%), conduct problems (28,2%), peer problems (11.8%), total difficulties (15.3%), and prosocial behavior (1.2%). History of sexual abuse was significantly associated with total difficulties, conduct problems, and peer problems.

Conclusion: There were emotional and behavioral problems among street children. History of sexual abuse was associated with behavioral problems. Therefore, more attention to this factor is needed to overcome the behavioral problems.

PUBLIC HEALTH

The Development of a Patients Concerns Inventory (PCI) as a Means of Improving Out-Patient Consultation and Patient Empowerment

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Introduction: The purpose of the Patients Concerns Inventory (PCI) is to allow patients to formulate an individualized profile of their concerns, needs and priorities that can be used as a structure to help guide out-patient consultations and promote multidisciplinary care. The PCI covers a range of issues including hearing, intimacy, fatigue, financial/benefits, PEG tube, relationships, regret, spiritual/religious aspects, support for my family, weight, wound healing.

Material and Methods: On attending the oncology out-patient clinic patients complete the 45-item PCI using a touchscreen computer (TST). Patients are asked to identify any concerns that they wish discussed and this data is networked into the consultation room.190 PCIs were completed by 135 patients over a 27 week period of one consultant from 1st August .The median time to complete the TST was 8 minutes. The vast majority found the technology no problem to use.

Results: The most frequently selected by patients were fear of recurrence (38% of patients), dental health/teeth (27%), chewing (24%), pain in head and neck (21%), swallowing (19%), and saliva (18%).The two MDT members they wished to see in clinic or be referred were the dentist (18%), and speech and language therapist (10%).The vast majority felt the PCI made a difference (quite a bit /very much) to their consultation as it made it 'a bit more personal', 'reminds them of the points they want discussed', 'allows the consultation to get straight to the point'.

Although the PCI has the potential to raise a lot of issues its use did not noticeably prolong the consultation.Median duration of consultation with PCI was 8 minutes and without PCI the median was 7 minutes.

Conclusion: Following this very successful pilot the PCI is being rolled out to other consultants in the Head & Neck clinic.

PUBLIC HEALTH

Movies & Medicine: Measuring the Impact on Interest Level

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Introduction: Combining a movie on a medical topic and an expert-led discussion on the topic of the film is increasingly being used as a tool for medical education. However, to date little is known about the impact of this combination on students' level of interest in the specific medical topic(s) discussed.

Material & Methods: This study aimed to assess the effectiveness of such movie-discussion combinations by measuring changes in students' perceived level of interest in three distinct medical topic(s) using a 5-point numerical scale. Three movie events were organized during which medical students watched a movie on a medical topic and then participated in a 30-minute discussion led by an expert. Movie 1 'As Good As It Gets' was shown in Event 1 (n=22) followed by a discussion on obsessive-compulsive disorder, while Movie 2 'Supersize Me' was shown in Event 2 (n=26) followed by a discussion on the role of nutrition in disease prevention. In Event 3 (n=25), Movie 3 'Sicko' was shown before a discussion on health and human rights. All events were approximately one month apart. Changes in perceived level of interest before and after each event were analyzed using the Wilcoxon matched-pairs signed-ranks test.

Results:

Event 1 (n=22, p=0.021)

Changes in interest level score	-4	-3	-2	-1	0	+1	+2	+3	+4
Frequency	0	0	0	2	10	7	2	1	0

Event 2 (n=26, p<0.001)

Changes in interest level score	-4	-3	-2	-1	0	+1	+2	+3	+4
Frequency	0	0	0	0	13	11	2	0	0

Event 3 (n=25, p<0.001)

Changes in interest level score	-4	-3	-2	-1	0	+1	+2	+3	+4
Frequency	0	0	0	0	8	9	7	1	0

Conclusion: A statistically significant increase in perceived level of interest after each movie and discussion was observed. The p value is more significant when larger numbers of students are involved. It can be hypothesized that combining a movie on a medical topic with an expert-led discussion on the topic of the film is effective in stimulating interest among medical students. As perceived interest has a major impact on students' learning and subsequent achievements, this movie-discussion combination may be a highly effective method for teaching Medicine.

PUBLIC HEALTH

Effect of Noise Stress on Serum Level of Sex Hormones in Adult Male and Female Mice

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Introduction: Noise has long been known as a health risk not only for workers of different industries but also for the general population. Several studies have proven the psychological, physiological and behavioral impact of noise stress but few is known about the mechanism of those impacts. Alteration in hormonal regulation might be the cause.

We already know from the previous studies that noise, as a stressor, increases cortisol and noradrenaline release but little is known about the effect of noise stress on sex hormones considering the fact that sex hormones have a variety of effects in human body.

The aim of the present study was to determine the effect of noise stress on the serum level of sex hormones and to investigate the sex related differences in response to noise stress.

Material and Methods: Male and female mice of the experimental groups were exposed to 100 dB rock music 2 hours per day for 7 days. Control groups were kept in a quiet environment. Other environmental factors such as humidity, temperature, light and size of the cages were the same for both control and experimental groups. Serum levels of testosterone, estrogen and progesterone were then assessed.
Results: 2 hour per day exposure to 100dB rock music for 7 days resulted in a significant decrease in estrogen level in female mice (3.56 versus 7.68, $p<0.01$) but did not have significant effect on neither progesterone nor testosterone level.

Conclusion: The results of this study indicate that noise stress has more effect on female sex hormones than male ones. Considering the role of estrogen in protecting women from many diseases such as Ischemic heart disease, osteoporosis and Alzheimer's disease, the findings of this research raise serious concerns about the effects of noise pollution on women's health. Further studies will focus on the exact mechanism by which noise stress decreases estrogen level.

We also believe that it is of great importance that noise protectors are used by women working or living in areas with high noise pollution level although the efficacy of such devices should be examined in further studies.

PUBLIC HEALTH

Body Mass Index and Blood Pressure in Population of Iran

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Introduction: To elucidate the correlation between body mass index (BMI) and hypertension/blood pressure in subjects with different levels of fasting plasma glucose (FPG) and also in different sexes.

Material and Methods: Our data are based on the results of the first Survey of Risk Factors of Non-Communicable Diseases of Iran, 2005. In this national cross-sectional survey 70,981 Iranian citizens (44,960 men and 44,178 women; age 15-65 years with mean ± SD of 39.0 ± 14.4 years) were recruited. Participants were interviewed and examined to determine clinical characteristics including systolic blood pressure (SBP), diastolic blood pressure (DBP) and BMI. FPG was also measured. 5,412 of participants were excluded because of taking antihypertensive drugs. Subjects with FPG ≥ 126 mg/dl were regarded as diabetics, those with 110mg/dl≤FPG<126mg/dl were designated as having impaired fasting glycaemia.

Results: Hypertension and blood pressure increased significantly with increasing BMI throughout the range of FPG. The odds ratio (OR) for hypertension for every 1 kg/m2 increment of BMI was 1.126 (1.120-1.132) and 1.105 (1.100-1.111) for men and women, respectively. In both sexes together, odds ratio for participants with normal FPG, those with IFG and diabetics were 1.077 (1.072-1.082), 1.072 (1.055-1.090) and 1.060 (1.044-1.076) respectively. For every 1 unit BMI increase, the SBP and DBP significantly increased by 1.063 and 0.655 for men; and by 1.010 and 0.634, respectively, for women. The regression coefficient for SBP estimated by BMI for participants with normal FPG, those with IFG and diabetics were 0.737, 0.745 and 0.679, respectively; and for DBP, it was 0.463, 0.487 and 0.419, respectively.

Conclusion: BMI is significantly linked to hypertension/blood pressure throughout the range of FPG and also in both sexes. The relative risk for hypertension by increasing 1 kg/m2 in BMI was higher in men (12.6%) in comparison with women (10.5%). For every 1 unit BMI increase, increment of SBP was more than DBP. This result shows that weight loss has more impact on SBP than DBP. The relation between BMI and hypertension/blood pressure was weaker in participants with diabetes than others, probably because many of diabetic patients were advised somewhere to control their blood pressure by changing lifestyle.

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PULMONOLOGY

Comparison of Allergy-related Biomarkers Between Allergic Rhinitis only and Combined Allergic Rhinitis and Allergic Asthma

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Introduction: For centuries, allergic rhinitis (AR) and allergic asthma (AA) have been considered separate disease entities with different diagnostic approaches and treatment modalities. In the second half of the 20th century, the phenomenon of the 'allergic march' was issued. Subsequently, many similarities have been shown between both diseases. This has led to the concept of 'one allergic airways disease' and the implications for one diagnostic and therapeutic approach have been summarized in the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines. However, despite the integrated concept, there are several differences suggestive of distinct phenotypes: allergic rhinitis only (AR-only), allergic asthma only (AA-only) and combined allergic rhinitis and allergic asthma (AR&AA). Primary objective: to identify potential differences in allergy-related biomarkers between allergic rhinitis only (AR-only) and allergic rhinitis and allergic asthma (AR&AA). Secondary objective: to explore the relationship between nasal nitric oxide (nNO) production and nasal eosinophils in nasal brushings (NAB eosinophils), on the analogy of the correlation between exhaled nitric oxide (eNO) and sputum eosinophils in the lower airways of patients with allergic asthma.

Material and Methods: A post-hoc analysis of baseline data of twenty subjects (19-50 years), participating in a biomarker validation study with clinically stable allergic rhinitis was performed. Ten of the subjects had concomitant, clinically stable, allergic asthma. None were current smokers or using controller therapy. Data comprised of allergy-related biomarkers obtained by skin prick test (SPT - wheal grass; trees; house dust mite), blood sampling IgE (BsIgE - grass; trees; house dust mite), nasal lavage (NAL – ECP α2-macroglobulin; IgE; MUC5AC), nasal brush (NAB - eosinophils) and nasal nitric oxide (nNO) measurements. Differences between groups were tested using a Wilcoxon rank test and the Spearman's rank correlation between nNO and NAB eosinophils was calculated.

Results: As compared with AR&AA, patients with AR-only showed a statistically significant higher wheal response to grass ($P = 0.006$), higher BsIgE-value to grass ($P = 0.038$), lower BsIgE value to house dust mite ($P = 0.049$) and an overall lower number of allergies ($P = 0.017$). There were no significant differences in other biomarkers between the two study groups. Furthermore, no significant correlation was found between nNO and nasal eosinophils recruitment ($r = -0.222$, $P = 0.426$).

Conclusion: Despite limited sample sizes, this post-hoc analysis points towards distinct immunological profiles in the AR-only and AR&AA phenotypes, and hence supports and extends existing data warranting further research with preferably higher patient numbers.

PULMONOLOGY

Detection of Mutations in Isoniazid Resistant *Mycobacterium Tuberculsosis* Isolates from Tuberculosis Patients in the Philippines

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Introduction: Drug-resistant tuberculosis is a significant clinical and epidemiologic dilemma, with the burden of disease reaching 8 million new cases reported annually and 1.7 million deaths per year. Resistance to first-line drug isoniazid (INH) threatens management and cure. INH resistance has been determined to involve the katG gene which converts INH to its bioactive form as well as encodes for catalase-peroxidase. This study identifies the mutations in katG found in isolates from the Philippines and describes them against findings elsewhere in the world.

Material and Methods: Thirty seven clinical isolates of Mycobacterium tuberculosis were collected from the Philippine General Hospital. Phenotype referencing, as well as antimicrobial drug susceptibility testing (AMST) was performed previously using CDC standard conventional proportional method. Segments of the katG gene were amplified and the PCR products were sequenced and compared with standard strains of H37Rv (ATCC 25618).

Results: The results confirm that the majority of mutations in the katG gene can be ACC mutation◊located in codon 315, and that the most common mutation is an AGC Thr). The results also show a disparity between the number of◊(Ser phenotypically resistant strains to isoniazid (89.2%) and the number of actual mutations located in the katG gene (59.4%), pointing to genes other than katG which may confer resistance. The study is consistent with global studies that show significant mutations in codon 315. The Philippine isolates, however, harbor few to no mutations in other codons relevant in other countries. The absence of mutations in codon 309, for instance, show differences in mutation patterns. A mutation in codon 333 (from Leu to Arg) is described by this study, a novel mutation not seen in other countries. There are also no multiple mutations found in a single strain in Philippine isolates, a prominent feature of mutations in Europe, particularly in Belarus, suggesting a possible lowered secondary infection rate in TB patients in the Philippines.

Conclusion:The study showed differences in the mutation profile of INH resistant strains in the Philippines compared to strains from around the world. The results also suggest that there are genes other than katG that also confer resistance in Philippine strains. The results, together with the absence of multiple mutations and deletion of the katG gene, show differences in mutations in Philippine isolates and the need to study the entire katG gene to identify significant mutations, which may aid in more rational and targeted drug use.

PULMONOLOGY

Measurement of Interferon Gamma Level in Yuberculosis Pleural Effusion

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Introduction: Even though tuberculosis pleural effusion is relatively uncommon in the United States, in some parts of the world it is endemic. In our country the most common cause of exudative pleural effusion is tuberculosis pleural effusion. The diagnosis can be established by culture of the pleural fluid, needle biopsy of the pleura or thoracoscopy, with the last two being very invasive procedures. Alternatively the diagnosis can be established by demonstrating high IeveIs of TB markers in the pleural fluid, Like Adenosine deaminase, interferon-y or positive PCR for tuberculosis DNA. Objective of this study was to investigate the diagnostic value of interferon γ (INF-γ) in tuberculosis pleural effusion.

Material and Methods: The patient enrolled in this study consisted of 20 patients with TB pleural effusion and 32 with Non – TB pleural effusion .Demographic data and characteristics of pleural effusion chemistry were compared in two groups.

Results: Demographic data, including age, sex, Job & nationality were almost the same in both groups. Dyspnea was the most common presenting symptom in both groups. Most of our patients were non smoker & PPD negative. Pleural effusion was yellow in both groups and Cholesterol and Triglycerides level were not significantly different. Low glucose and high protein levels were found in tuberculosis plural effusion. There was no significant difference in lymphocyte and Neutrophil count between two groups. INF-γ was significantly higher in Tb pleural effusion (P=0.001).

Conclusion: INF-y level is a valuable and non invasive diagnostic method for TB pleural effusion especially in cases which you are in doubt about the diagnosis. But as it is not cost- effective so we do not recommend it as a routine diagnostic method in assessment of all pleural effusion.

PULMONOLOGY

Survival in Patients on Long-Term Oxygen Therapy

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Introduction: Long-term oxygen therapy (LTOT) is the only treatment improving survival of patients with respiratory failure due to chronic obstructive pulmonary disease (COPD). However, LTOT is recommended in all chronic respiratory diseases with severe hypoxaemia despite lack of evidence of efficiency. The aim of the study was to analyse survival in patients qualified to LTOT, to compare survival between COPD patients and other respiratory diseases and to reveal factors which correlate with better prognosis in COPD patients.

Material and Methods: Consecutive patients qualified to LTOT in 2004-2007 were included. Eligibility for LTOT was based on the ATS/ERS guidelines. The data concerning anthropometric parameters and lung function were taken from medical charts of the patients. Mortality was evaluated over time using Kaplan-Meier analysis.

Results: Study group consisted of 80 patients (72% with COPD, 53% female) aged 69±10 years, mean FEV1 44±20 % pred., RV%TLC 65±14%, PaO2 49±6 mmHg, PaCO2 45±10 mmHg, dyspnea scale MRC 3,9±1. There were 53 (66 %) deaths during the study, with a median survival time 31.4 months. The overall mortality rate was 72% in the COPD group as well as in non-COPD group. There were no differences in survival time between COPD patients and patients with respiratory failure caused by other diseases (i.e. restrictive chest wall disorders, interstitial lung diseases, pulmonary hypertension and others). High PaO2 (p=0,01) and VC % pred. (p=0,02) were the only measures significantly correlated with increased survival in COPD patients on LTOT.

Conclusion: The prognosis of patients requiring LTOT is poor. The mortality rate was equal in the COPD and non-COPD group. Higher VC % pred. and PaO2, seemed to predict better prognosis in COPD patients treated with LTOT.

PULMONOLOGY

Allergy to Fungi in Patients with Bronchial Asthma

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Introduction: Establishing the frequency of allergy to fungus in patients with bronchial asthma and finding a correlation between intensity of asthma symptoms and frequency of allergy to fungal moulds.

Material and Methods: The research was carried out in 276 patients (171F and 105M) aged 5-68 treated in the Outpatients Clinic of Allergic Diseases. Every patient was interviewed for allergy history. Every patient had:
1. Spirometry at rest and bronchodilatatory test
2. Skin-Prick test for common fungal allergens.
3. We started immunological diagnostic of patients (total IgE and specific IgE), first results for 68 patients.

Results: All patients suffered from bronchial asthma, 133 patients (48,19%) from mild form of asthma, 75 (27,17%) had FEV1 from 60% to 80% of norm in the spirometry and 68 (24,64%) had FEV1 below 60% of norm. Positive skin prick test for Alternaria in 22 cases (7,97%), Aspergillus in 23 cases (8,33%), Cladosporium in 21 patients (7,61%). Positive skin prick test for at least one fungal allergen in 45 patients (16,3%). Immunological results: increased level of total IgE in 32 patients out of 68 (47,06%) and increased level of specific IgE for Alternaria in 7,3% of patients, Aspergillus in 10,3% of cases and Alternaria in 10,3% of patients. We were searching for a correlation between severness of asthma symptoms and the frequency of positive skin prick tests for fungal allergens. In the group with mild asthma positive skin prick tests for Alternaria were found in 10 patients (7,52%), in the group with FEV1 between 60-80% of norm in 9,33% and in patients with FEV1 below 60% of norm in 7,35% of patients ($r=-0,03$; $p<0,05$). In the group with mild asthma positive skin prick tests for Cladosporium were found in 7,52% of patients, in the group with FEV1 between 60-80% of norm in 10,67% and in patients with FEV1 below 60% of norm in 4,41% of patients ($r=0,10$; $p<0,05$). In the group with mild asthma positive skin prick tests for Aspergillus were found in 6,77% of patients, in the group with FEV1 between 60-80% of norm in 14,67% and in patients with FEV1 below 60% of norm in 4,41% of patients ($r=0,12$; $p<0,05$).

Conclusion:
1. We found no statistical evidence of correlation between intensity of asthma symptoms and frequency of allergy to fungal moulds.
2. In the population considered in the research 16,3% had positive skin prick tests for at least one fungal allergen.

RADIOLOGY

Optimization of Clinical Functional MRI Protocols at 3 Tesla

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Introduction: Functional MRI (fMRI) methods are integral steps of pre-surgical workup nowadays, but the protocols applied differ across imaging centers. Moreover, the majority of paradigms have been validated on 1.5T, while the better signal-to-noise ratio and the higher spatio-temporal resolution of higher field scanners can yield different maps for similar paradigms. Therefore, we aimed to evaluate a set of paradigms for mapping language production and comprehension, motor cortex, and hippocampus while defining optimal acquisition parameters, as well.

Material and Methods: We performed functional MRI on 17 healthy volunteers in a block design manner using picture naming, grammatical decision, auditory comprehension, auditory grammatical decision, cued motor response, and spatial memory paradigms. A parametric evaluation of statistical significance versus paradigm length followed, by modifying the number of paradigm blocks involved in processing in order to find the shortest paradigm length providing solid results. Moreover, we compared the configuration of functional activations yielded by the four different language mapping paradigms.

Results: All of the investigated language, motor and memory mapping paradigms provided solid and consistent activation maps comparable with the literature. The auditory grammatical decision paradigm was capable of mapping language production and comprehension networks in one step. The configuration of language-related activation maps differed across paradigms within the same functional areas depending on the task and the number of stimulus blocks. We found that four active blocks can already be enough for defining the centre of gravity of activations, but including more blocks in the calculations increase the complexity of the obtained maps. Interestingly, if the number of blocks reaches 9 the variability of the maps starts to decrease. Moreover, lateralization indices also depended on the number of blocks: lateralization tended to decrease if more than 6 blocks were included in the model. The cued motor response task yielded activations as expected from the literature, while the investigated memory paradigm activated the anterior and posterior hippocampus in a different manner.

Conclusion: Using more paradigms for language mapping improves the outcome of the fMRI examination; moreover, with tackling different aspects of language it also provides an opportunity for a finer grain identification of processing steps. Based on our results, six repetitions of the active block seem to be the optimal choice for mapping, having the smallest impact on lateralization calculations, and a relatively low variability in the maps.

The tested memory paradigm needs to be reworked.

RADIOLOGY

Multislice Computed Tomography in the Assessment of Severity and the Course of Acute Pancreatic Necrosis

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Introduction: We researched the prognostic value of multislice computed tomography (CT) in the assessment of severity of acute pancreatic necrosis. The term of acute pancreatic necrosis was used as a synonym for severe forms of acute pancreatitis in this study. The aim was to define radiological parameters of pancreatic necrosis according to the localization and expansion of the pathological changes. And to define their correlation with the clinical state and the course of the disease.

Material and Methods: The study was performed on 34 patients who underwent contrast enhanced multislice CT within maximally 72 hours after onset of symptoms in the first episode of the disease. Patients were divided in groups using the radiological findings based on defining: the CT grade of acute pancreatitis, the necrosis score and the CT severity index. The results were statistically processed.

Results: The distribution of patients by CT grade shows that the largest number of patients belongs to the group E (41.17%). According to the necrosis score, the largest number of patients (64,71%) belongs to the group I where pancreas is uniformly shown after the appliance of contrast. According to the complications, we found no significant difference comparing CT grade and necrosis score with the frequency of complications. We found statistically significant difference comparing appearance of complications with the CT severity index.

Conclusion: The appearance of complications depends on the value of the CT severity index. Multislice CT examination is the most reliable method to define the presence and predict the course of pancreatic necrosis.

RADIOLOGY

Assessment of Thrombosis Using Strain Echocardiography in Patients with Persistent Nonvalvular Atrial FSibrillation

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Introduction: The incidence of thromboembolism remains high in patients with atrial fibrillation (AF). The left atrial appendage (LAA) is a potential site for development of thrombus and LAA dysfunction is an independent predictor of thromboembolism. The magnitude of LAA flow velocities are dependent on acute changes in loading conditions. Myocardial velocities obtained by tissue doppler imaging are less dependent on preload and may help in more accurate risk prediction.

This study was planned to assess whether LAA longitudinal strain (LAAS) is a useful method for prediction of the LAA thrombosis in patients with nonvalvular persistent AF.

Materials & Methods: We studied 52 patients with nonvalvular persistent AF referred for clinically indicated echocardiographic study. Patients undergoing anticoagulant therapy were excluded. Left atrial diameters (LAD), surface (LAS) and volume (LAV) were measured by using transthoracic echocardiography. LAA emptying (LAAEV) and filling (LAAFV) velocities were measured by transesophageal Doppler echocardiography. Real-time 2-dimensional color Doppler myocardial imaging data were recorded from the LAA at a high frame rate. LAAS was performed with offline analysis from midsegment of lateral wall of LAA. LAAEV, LAAFV and LAAS were averaged for five consecutive cardiac cycles.

Results: Transesophageal echocardiography demonstrated LAA thrombus in 20 of 52 patients; in all of these patients the spontaneous echo contrast was detected. The area under the receiver-operating characteristic curve (ROC) for predicting the patients with LAA thrombus was greatest for LAAS (AUC=0.92, p<0,001) (figure 1), followed by LAAEV (AUC=0.86, p<0.001), LAAFV (AUC=0.85, p<0.001), LAV(AUC=0.70, p<0.001), LAS (AUC=0.69, p<0.001) and LAD (AUC=0.66, p<0.001). The differences between the ROC curves for LAAS and LAAEV or LAAFV are statistically significant (p<0.05). The differences between the ROC curves for LAAEV and LAAFV are not statistically significant (p>0.05). The optimal LAAS cut-off for predicting the patients with LAA thrombus was 2.95 % (sensitivity of 93% and specificity of 80%). For LAAEV the optimal cut-off was 22.5 cm/s (sensitivity of 86% and specificity of 79%).

Conclusion: The present results suggest that LAAS characterizes LAA function and might serve as a predictor of thrombus formation in nonvalvular persistent AF. LAAS appears to be a clinically applicable and reliable imaging technique, and may complement flow velocities in evaluating LAA function for risk assessment of thromboembolism.

RADIOLOGY

Validation of Mandibular Radiomorphometric Indices to Identify Reduced Skeletal Bone Mineral Density in Post-menopausal Women

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Introduction: Osteoporosis is the most common metabolic bone disease and characterized by an increased risk of bone fractures. Women are more commonly affected than men. Early detection of osteoporosis can prevent the hip fracture late in life. The purpose of this study was to evaluate the radiomorphometric indices of the mandible on panoramic radiographs in normal, osteopenic and osteoporotic post-menopausal females.

Material and Methods: An assessment of nine indices included Mental Index (MI), Panoramic Mandibular Index (PMI), mandibular crest resorption degree (M/M ratio), Mandibular Cortical Index (MCI), gonial angle, antegonial angle and depth, Antegonial Index (AI) and the number of mandibular teeth, were performed on dental panoramic radiographs of post-menopausal women (age range; 45-74 years) with two observers. Bone Mineral Density (BMD) at the lumbar spine was measured by Dual Energy X-ray absorptiometry. The World Health Organization (WHO) criterions of T-score were used to diagnose the normal patients, patients with osteoporosis or osteopenia.

Results: In this study, there were no statistically significant differences between the three categories of skeletal bone status for PMI, M/M ratio, gonial angle and the number of mandibular teeth. The antegonial angle, AI and MI were significantly smaller in individual with low bone mass ($p<0.05$). The antegonial depth was significantly greater in osteoporotic individuals ($p<0.05$). BMD results showed significant difference among the three subgroups of MCI. Inter and intra observers' agreement was good to excellent. Multivariable logistic regression analysis used the antegonial index and antegonial depth to generate the following equation for probability of having osteoporosis for a new subject:
$Z=5.4+ [0.8\times\text{antegonial depth}] - [1.8\times AI]$.

Conclusion: Osteoporotic individuals are more likely to have altered inferior cortex and antegonial region morphology and thickness than non-osteoporotic individuals. The smaller antegonial index and greater antegonial depth were significantly associated with lower bone mass.

RADIOLOGY

Testing of Global Histogram Equalization and Unsharp Mask Algorithms for Processing of Conventional Chest X-ray Images

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Introduction: We designed a windows-based computer program that contains histogram equalization (HE), unsharp (UM) mask and combination of HE and UM algorithms with adjusted parameters to process conventional chest x-ray (CXR) images. We converted conventional chest radiographies to digital images by means of a digital camera. The aim of this research was to evaluate the effect of digital image processing on conventional CXR images.

Material and Methods: Two series of CXR images were selected which contain 49 images without major pulmonary disorder and 45 images with major pulmonary disorder, respectively. After converting to digital format, images were processed with HE, UM and combination of HE and UM techniques. In each series, primary and processed images were saved in 4 databases. Two board-certified general radiologists (with 6 and 5 years experience) analyzed images. Saved images were displayed to radiologists randomly and separately. Quality of each image was saved as a scale from 1(very low quality) to 5(excellent). We used a variance-based statistical technique to analyze the quality.

Results: In the first series of images, HE and combination of HE and UM algorithms increased quality of images, but UM technique was not suitable, solely. Also, all three techniques increased quality of second series of images.

Conclusion: Low-quality imaging due to inappropriate radiation, eliminated devices and patient's condition lead to reimaging and unnecessary exposure. The use of digital image processing algorithms on conventional CXR images can increase quality and therefore reduce reimaging, exposure and elimination of devices.

SURGERY AND ORTHOPAEDICS

The Darrach Procedure for Post-Traumatic Reconstruction

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Introduction: The Darrach procedure was once a common procedure for distal radioulnar joint (DRUJ) dysfunction, but has fallen out of favor recently, due to concerns about residual pain and instability of the ulna. Our impression is that the Darrach resection of the distal ulna continues to be a successful post-traumatic reconstructive procedure, particularly when the indication is not solely pain relief, but rather stiffness, instability of the distal radioulnar joint, or substantial radioulnar length discrepancy.

Material and Methods: Over a 6-year-period, 26 patients (15 female, 11 male, mean age 53 years [range 24 to 80 years]) were treated with Darrach's procedure for reconstruction of post-traumatic distal radioulnar joint dysfunction. The indication for Darrach's procedure was substantial restriction of forearm rotation in 16 patients, substantial radioulnar length discrepancy in 5 patients, chronic DRUJ instability in 2 patients, nonunion of the distal ulnar diaphysis in 2 patients, and ununited distal radioulnar arthrodesis (Sauve-Kapandji procedure) in one patient. Average pre-operative arc of forearm rotation was 49° (range, 0° to 160°) and average arc of wrist flexion and extension was 55° (range, 20° to 140°). Six patients reported rest pain and 10 reported pain with some activities of daily living.

Results: At an average follow-up of 21 months (range, 4 to 60 months), improvement in total arc of forearm rotation averaged 87° (range, 0° to 160°; $p < 0.001$) for an average final arc of rotation of 136° (range, 20° to 160°). Average improvement in total wrist flexion-extension arc was 23° (range, -15° to 90°; $p = 0.004$). The number of patients with occasional or continuous pain after the Darrach procedure (7 after vs. 16 prior; $p = 0.04$) was significantly reduced. There was 1 patient who demonstrated chronic radioulnar impingement on radiographs, but additional treatment was not requested.

Conclusion: The Darrach procedure improves forearm rotation and pain in patients with posttraumatic distal radioulnar joint derangement, with a low complication and re-operation rate.

SURGERY AND ORTHOPAEDICS

A New Fixator of Small Animals Diaphyseal Fractures

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Introduction: In the experiment the effect of the new rod-through-plate fixator on the fracture healing has been investigated. The fixator is made of stainless steel and consists of a support plate, two curved rods and two cortical bone screws. The new method aims to maximally reduce the traumatization of soft tissues during the operation and ensure strong fixation of bone fragments.

Material and Methods: Experimental bone fractures were induced in the central third of the diaphysis of the five sheep's tibia. The animals were subdivided in the two groups: group I – three animal with rod-through-plate fixator; group II – two animals with plate fixator. The regeneration of the bone tissue was studied using clinical, biochemical, radiographical and morphological methods. The animals were in the experiment for 10 weeks.

Results: In the group I animals the functional recovery of the operated limb was considered good 4 days after the surgery; in the group II – 7 days after surgery. The new fixator did not exhibit any adverse effect on the bone structure with follow-up radiological evaluations and fixators remained in the same position as originally applied. The fixator was removed 4 to 8 weeks after surgery. The bone fracture areas were studied by means of histological and immunohistochemical methods. Weak osteocalcin and osteopontin expression was seen 2 weeks after beginning of experiment in the biopsy material. Collagen II expression was high already on the second week, but still increased at week 8. Results with active periostal bone tissue formation and decreased amount of cartilage tissue (8-10%) in callus support the beneficial effect of the new fixator on the fracture healing.

Conclusion: The new bone fixation method for diaphyseal fractures of long bones allows to lengthen the shoulder of the rod-through-plate fixator and by that to reduce pressure in the area of the fracture, to ensure stability and normal fracture healing. Furthermore, the traumatisation of soft tissue during operation is minimal and the operated limbs are fully weight bearing.

SURGERY AND ORTHOPAEDICS

Determination of Geometric Indices of the Femoral Bone and its Association with Bone Density of Proximal Femur

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Introduction: Geometric indices of the femoral neck and bone mineral density (BMD) have been proposed to have a major role in prediction of risk of hip fracture. Some studies have shown a racial variation in such indices. Meanwhile, no previous study in Iran has estimated these parameters for Iranian patients. Moreover, no study has inspected the relationship between these indices and BMD parameters in different sub-regions of hip.

Material and Methods: Postmenopausal women referred to the bone densitometry center of the Shariati Hospital comprised the study population. Patients with apparent risk factors of osteoporosis were excluded. Bone mass was determined using dual-energy X-ray absorptiometry on Lunar DPX bone densitometers. For calculation of femoral neck geometric indices, the printout of the hip densitometry with magnification of ½ was used. Hip axis length (HAL) and neck-shaft angle (NSA) were the main outcomes of the study. We used Pearson Correlation analysis for determination of relationship between geometric indices and other variables and multivariate linear regression analysis for adjustment of other independent variables in prediction of femoral BMD.

Results: Among 103 postmenopausal women studied, mean HAL was 99.8 ± 4.3 millimeters and mean NSA was 120.3 ± 4.3 degrees. In Pearson Correlation analysis, there was no correlation between HAL and NSA with BMD results of different sub-regions of hip and there was just a significant positive correlation between HAL and height and weight. Results of multivariate regression analysis also revealed no significant relationship between HAL and NSA with BMD of total femur and femoral neck.

Conclusion: According to our results, it is unlikely that geometric indices have a major role in determination of hip BMD parameters and so their routine use in bone densitometry centers is not supported. Mean HAL of Iranian women is lower than that of Western countries and higher than that of East Asian countries. This is in line with observation of rates of hip fracture in Iran lower that Western rates and higher than Eastern rates.

SURGERY AND ORTHOPAEDICS

Repairing Traumatic Inferior Orbital Wall Fractures Using Nasoseptal Cartilage Graft

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Introduction: The goals of reconstruction in orbital fracture are to restore floor continuity, provide support of orbital contents, and prevent fibrosis of soft tissues. Nasoseptal cartilage is an easily accessible, abundant, and autogenous source that supports orbital floor and minimal donor site morbidity. This study evaluated the effectiveness of nasoseptal cartilage for repairing traumatic orbital floor defects.

Material and Methods: Autogenous nasoseptal cartilage was used in 20 patients. Presence or absence of diplopia, enophthalmus, infraorbital nerve paresthesia, dystopia, range of covering of the defect by nasoseptal cartilage, complication of recipient and donor site, resorption of graft, and ocular mobility disorders were evaluated. Entrapment of orbital tissues, large orbital defect (greater than 50% of orbital floor or more than 8 mm), or orbital floor defects with involvement of other zygomaticofrontal complex fractures are indications for surgical orbital exploration. In one case after 24 months, the surgical field was explored for direct evaluation of the grafts' efficacy.

Results: All patients were successfully treated by restoration of the orbital floor wall continuity. Six months to 2 years follow up showed only one patient with postoperative enophthalmus. There were no donor site morbidity and graft infections or extrusion. The nasoseptal graft was completely covered with underlying tissue.

Conclusion: Nasoseptal cartilage is a readily accessible autogenous tissue that should be considered when an autogenous graft is needed for reconstruction of orbital floor defect.

SURGERY AND ORTHOPAEDICS

Influence of Surgical Procedures on the Cognitive Function and Quality of Life in Patients Undergoing Open Heart Surgery

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Introduction: As a complication of surgical procedures and anesthesia during cardiac surgery impairment of the nervous system by among others ischaemia can occur. The appearance of deterioration of the postoperative cognitive function as a result of surgical intervention on the heart is proved by many studies. It can have multiple reasons: perfusion disorder in the brain, micro air embolism, tissue embolism, etc. these could be responsible for the appearance of the cognitive disorders together. The focal and/or diffuse neuronal damage developed in brain appears in a form of cognitive function disorder but may also can cause a permanent decrease in function.

Material and Methods: Up to now we analysed 41 patients (mean: 57±9.6 years, SD: 32-78 years; man/woman: 78 % vs. 22 %) pre- (before operation [T0]) and two times post (5.-6. postop. day [T1-early postop. shadowing] and 4.-6. postop. month [T2-long term shadowing]) cardiac operations. The major fraction of the vetted patients (24 patients [60 %]) had coronary intervention, the others (17 patients [40 %]) had valve operation, or combination of them.

We realized our scrutiny based on the result of psychological tests (filled out independently: SF-36, HADS) and neurocognitive tests (filled out controlled by ourself: Digit Span [DSp-F and B]; Auditory Verbal Learning Test [AVLT]; Trail Making Test [TMT-A and B]; Verbal Fluency; Rey Complex Figure; Pieron Test). We defined 4 independent dimensions with factor analysis of 18 neurocognitive variables: verbal fluency, verbal learning, visual memory and attention, working memory. To use these we identificated the patients at who we recorded an impairment with more than 1 SD.

Results: Based on our results we can tell, that there is a registered improving at T2 time in the concern of some cognitive abilities (TMT-B, DSp-F, DSp-B, AVLT). 22 % of our patients showed significant deterioriation at T1 and at T2. Patients with cognitive decay showed a tendentious worse quality of life and a higher anxiety level.

Conclusion: In the case of patients who had a cardiac operation – besides further monitoring cardiovascular functions – would be necessery to pay attention onto the other factors, that basically influence quality of life, hereby the complications could decrease.

SURGERY AND ORTHOPAEDICS

Prognosis of Hepatic Failure Development in Patients with Cirrhosis at Choice of Method of Surgical Treatment

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Introduction: Surgical method is basic in treatment of complications of portal hypertension. Hepatic failure is the most terrible and often complication of postoperative period. Lethality from the hepatic failure changes from 12.5 to 75%. The aim of the work: determination of risk factors and prognosis of hepatic failure development at choice of method of surgical treatment.

Material and Methods: In 124 patients with LC (liver cirrhosis) was performed the analysis of results of surgical treatment. Distal splenorenal shunt by Warren was performed at 81 patients, haemostatic suturing -at 40 patients. Indications to surgical treatment were ascites, hemorrhage from esophageal and gastric variceal veins. Biochemical, instrumental and morphological researches have been performed before the operation.

Results: It was established that hepatic failure occurs in 33.9% patients with LC and is a reason of their death in 63.3% cases. Highest risk of its occurrence is after distal splenorenal shunt at presence of ascites, combination of ascites and hemorrhage from esophageal and gastric variceal veins. Hepatic failure is accompanied by hypoproteinemia, hypoalbuminemia, increased concentration of bilirubin, urea, creatinine, hyperactivity of the aspartataminotransferase ,alaninaminotransferase, increase of anemia, leucocytosis, lymphopenia.The analysis of qualitative and quantitative characteristics of structural changes of the liver at LC has allowed to found out tree morphological types of LC(A,B,C) that reflects functional reserves of the liver. Decrease of the hepatic blood supply, linear portal velocity, increase in diameter of portal and splenic veins were observed at the patients with developed hepatic failure. Method of mathematical analysis allows to construct the equations of the prognosis of hepatic failure.

Conclusion: The highest risk of hepatic failure occurrence is after the distal splenorenal shunt at presence of ascites , combination of ascites and hemorrhage from esophageal and gastric variceal veins. Morphological changes in liver at LC reflect its functional reserves and risk of the hepatic failure is highest at C-type cirrhosis. Decrease of the hepatic blood supply, linear portal velocity ,increase in diameter of portal and splenic veins reflects decrease of functional liver reserves .Its possible the preoperative prognosis of the hepatic failure based on mathematical analysis of clinical , laboratory, morphological and instrumental parameters.

SURGERY AND ORTHOPAEDICS

Breast Augmentation: Comparison of Different Techniques

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Introduction: According to the American Society of Plastic Surgeons nowadays they perform 600% more breast augmentations than they did 15 years ago and the number is increasing. The variety of methods is increasing as well. Although we do not have the exact data about situation in our country but the tendencies are definitely the same. That is why it is important to know which method gains the advantage over the others. The aim of the study was to compare different techniques of breast augmentation by evaluating duration of the operation, hospital stays and postoperative complications.

Material and Methods: 381 patients underwent breast augmentation during the year 2006 to 2008 in the Plastic surgery center. 131 of them had chosen transaxillary incision: 95 submuscular placement and 36 subfascial placement of an implant; 142 had chosen priareolar incision: 41 submuscular 89 dual plane and 12 subfascial placement of an implant; 108 had chosen inframammary incision: 76 submuscular 23 dual plane and 9 subfascial placement of an implant. The duration of the operation, hospital stays and postoperative complications of each method were evaluated and compared.

Results: The average duration of the operation (minutes) using transaxillary submuscular technique was 55, transaxillary subfascial 62, periareolar submuscular 119, periareolar dual plane 115, periareolar subfascial 112, inframammary submuscular 93, inframammary dual plane 102, inframammary subfascial 174. The average hospitalization time (hours) using transaxillary submuscular technique was 50, transaxillary subfascial 47, periareolar submuscular 73, periareolar dual plane 86, periareolar subfascial 52, inframammary submuscular 72, inframammary dual plane 83, inframammary subfascial 49. There was 1 capsular contracture (1%) in transaxillary submuscular group; 1 capsular contracture (3.3%) and 2 seromas (6.6%) in transaxillary subfascial group; 2 capsular contractures (5%) and 2 (5%) seromas in periareolar submuscular group; 2 seromas (2.2%) and 3 haematomas (3.4%) in periareolar dual plane group; no complication in periareolar subfascial group; 1 capsular contracture (1.3%) and 3 seromas (4%) in inframammary submuscular group; 1 seroma (4.3%) in inframammary dual plane group; no complications in inframammary subfascial group.

Conclusion: The shortest operation time was achieved when incision had been made transaxillary and the implant had been placed submuscular. Patients spent least time in hospital when the implant had been placed subfascial transaxillary. There were no capsular contractures in dual plane group although it had some haematomas; also there were least seromas in submuscular group.

SURGERY AND ORTHOPAEDICS

Intraportal Injection of Autologous Porcine Mesenchymal Stromal Cells Augments Liver Regeneration after Portal Vein Ligation

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Introduction: Portal vein embolization (PVE) can be used prior to liver surgery to increase the volume of the remaining liver tissue after an extensive resection. However, the application of PVE is limited and new strategies to augment liver regeneration by cellular therapy are promising alternatives.

Material and Methods: We analyzed the influence of syngenic multipotent mesenchymal stromal cells (MSC) on liver regeneration after the ligation of the right portal vein branches in a porcine model, closely mimicking the situation of human surgery. N=15 piglets were included into this study (n=6 into the MSC-treated group, n=9 into the control group). Portal vein branches for the caudate, right lateral and right medial lobes were ligated. MSC were then applied into the non-occluded portal vein branches. Liver regeneration was monitored by ultrasonography, immunohistological analysis and serum biochemistry (bilirubin, urea, creatinine, alkaline phosphatase, gamma glutamyltransferase, cholinesterase, aspartate aminotransferase, alanine aminotransferase, albumin, C-reactive protein, cytokines TNF-α and IL-6, as well as growth factors HGF, TGF-β1 and IGF).

Results: The volume of the non-ligated liver lobe increased in all piglets after portal vein ligation. The growth acceleration of the hypertrophic liver lobes in the MSC group was maximal between the 3rd and 7th postoperative day ($p<0.05$ versus control). This stimulating effect was slowing down during the second week. However, the average volume of the non-occluded liver lobe was increased by 30% in the MSC group in contrast to the control group at the end of the experiment. Biochemical liver function was stable in all pigs. Only solitary transplanted MSC were detected in recipient livers two weeks after the infusion.

Conclusion: The infusion of porcine MSC into the portal vein in a setting of liver regeneration after surgical resection leads to accelerated and augmented hyperplasia. This effect is most likely due to bystander effects of the transplanted MSC.

VASCULAR MEDICINE

Age Related Functional Role of Concealed Slow AV Nodal Pathways During Atrial Fibrilation (AF); Revealing Role of IntermediateACC

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Introduction: The occurrence of atrioventricular (AV) nodal reentrant tachycardia (ANNRT) is age-dependent. This relation of age to occurrence of AVNRT is unexplained. Maturational changes in the conduction and refractoriness of the slow and fast AV- nodal pathway may explain the increased incidence of AVNRT with age.

The aim of this study was to obtain new insight into possible relation between age and functional electrophysiological properties of concealed slow pathway after ablation of fast pathway during experimental simulated AF.

Material and Methods: Selective stimulation protocols were used in two groups of neonatal (N=8) and young (N=8) isolated superfused AV-Nodal rabbits. To quantify the effects of FP ablation on the AV node, AF was simulated by random high atrial pacing. Small miniature lesions were made by delivering constant voltage(110 V-30 s) with 0.25mm diameter unipolar silver electrode .

Results: In control Nodal minimum conduction time and refractoriness (effective& functional) were shorter in neonatal compared to young rabbits. Selective FP ablation in young rabbits resulted in longer minimum nodal conduction time , but without change of nodal effective and functional refractory period.

Compact node ablation in neonatal rabbits prolonged minimum conduction time (47.7 ± 2.5 ms to 57.1± 3 ms , p< 0.0001) , maximum conduction time (113.1 ± 6.4 ms to 153.7 ± 13.5 ms , p< 0. 001) and functional refractory period (136.6 ± 4.1 ms to 148.8 ± 4.3 ms , p< 0. 01), but did not alter effective refractory period . The selective FP ablation in young rabbits resulting in little effect on the ventricular rate whereas in neonatal rabbits increased the average His-His interval (181.6±13 to 213.7±15 ms, P<0.0001). Zone of concealment increased significantly in the neonatal rabbits after FP ablation(16±9.3ms to35±11.5ms, p<0.01).Morphological evidence indicated that FP ablation damaged the transitional cells of the superior-anterior approaches of compact nodal cells.

Conclusion: During simulated AF, successful FP-ablation resulted in visualization of concealed slow pathway with different functional properties in neonate and young rabbits. Selective FP-ablation has significant effect on H–H intervals in neonatal rabbits, since newly manifest SP couldn't interact with slow pathway by intermediate accessory pathway. Dynamic of concealed slow pathway is more sensitive tool than the conduction curve to reveal the interaction between the two pathways in producing reentry. These findings indicate that input interaction is a major determinant of normal nodal properties.

VASCULAR MEDICINE

Aortic Flow Jet Angle Is An Indicator Of Aortic Root Dilatation In Bicuspid Aortic Valve Patients

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Introduction: Bicuspid aortic valve (BAV) is the most common congenital heart defect. BAV patients are prone to develop infective endocarditis, aortic stenosis and/or regurgitation, aortic root dilatation and aortic dissection. Current clinical methods poorly predict which patients will develop significant aortic root dilatation and thus fail to guide decisions for early medical or surgical intervention. The aim of this study is to analyze blood flow patterns in the aortic root of BAV patients by cardiac MRI and to determine whether (abnormal) flow patterns are an indicator for the development of aortic root dilatation.

Material and Methods: 19 asymptomatic BAV patients (14 male, 5 female, age 15.4 +/- 2.1) and 11 controls (7 male, 4 female, age 14.6 +/- 2.7) underwent a cardiac MRI. Structural images included a 4-chamber view, two orthogonal planes through the left ventricular outflow tract and a 'candy-cane' view through the aortic arch. 3-D velocity-encoded acquisitions were obtained in seven slices at, proximal, and distal to the aortic valve plane. Blood flow patterns were analysed with in-house HeartViz/FlowViz software, using combined 3-D visualization of blood flow and structural images. The aortic root channel axis at systole was calculated from border definitions in two orthogonal views through the aortic root axis. The direction of the systolic aortic flow jet through the valve plane was determined from 4-D velocity data. The angle between these vectors, a quantitative parameter of misdirected flow, was hypothesized to be associated with aortic root dilatation. Aortic diameter was measured at the aortic valve (AOV), sinuses of Valsalva (SoV), the sinotubular junction (STJ) and ascending aorta (AA).

Results: The average angle between the systolic aortic flow jet at the level of the aortic valve and the left ventricular outflow tract axis was found to be significantly larger in BAV patients (18.24 +/- 1.91 degrees) compared to control subjects (10.03 +/- 1.29 degrees, p=0.001). Aortic flow jet angle and aortic diameter at the level of the ascending aorta were significantly correlated in BAV patients (p=0.020), but not at the other levels. For BAV patients and control subjects combined aortic flow jet angle and aortic diameter were significantly correlated at the level of the SoV (p=0.023), STJ (p=0.014) and AA (p=0.001).

Conclusion: A significant correlation was observed between the aortic flow jet angle and aortic diameter. Further research is necessary to investigate whether there is a causal relationship.

VASCULAR MEDICINE

Carotid Artery Wall Shear Stress is Associated with Periventricular White Matter Hyperintensities and Stroke

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Introduction: Wall shear stress (WSS) is the frictional force exerted by the circulating blood on the endothelium. It has been shown that low WSS modulates the endothelium and in the long-term contributes to the development of atherosclerotic lesions. Carotid artery WSS (CAWSS) has been extensively studied. Previous studies demonstrated that CAWSS not only reflects the condition of the carotid artery itself, but that CAWSS can also be regarded as a summary of the systemic vasculature condition in terms of atherosclerosis. However, at present no studies have been performed exploring potential associations between CAWSS and neuroradiological markers of cerebrovascular pathology. In the present study we aimed to find such associations.

Materials & Methods: Quantitative cerebrovascular parameters (periventricular and subcortical white matter hyperintensities (WMH), count of stroke and brain atrophy) were derived from PD/T2 and FLAIR MRI, performed in 329 subjects (70-83 yrs, PROSPER baseline). CAWSS was derived from 3D paraboloid velocity data multiplied by viscosity, fitted by the Carreau-Yasuda model with individual hematocrit in the exponent. The cardiac cycle was divided into early-, mid- and end-diastolic and peak-systolic CAWSS. A linear regression analysis, adjusted for gender and age, was applied to investigate possible correlations between CAWSS and the presence of cerebrovascular pathology. Correlations with WMH were adjusted for intracranial volume as well.

Results: All associations were strongest with mid-diastolic CAWSS, followed by respectively end- and early diastole and weakest in peak-systole. All were significantly associated with periventricular WMH ($p=0.004 – 0.034$) but not subcortical WMH ($p>0.052$). Secondly, count of stroke was significantly associated with mid- and end-diastolic CAWSS ($p=0.016$; 0.026). Finally, CAWSS was not related to brain atrophy ($p>0.410$).

Conclusion: Interestingly, low CAWSS was associated with atherosclerotic related neuroradiological pathology such as periventricular WMH and count of stroke but not with parameters that currently have no association with atherosclerosis (subcortical WMH, brain atrophy). These findings may stimulate further research to extend even more the application and meaning of CAWSS.

The differences in results between diastolic and systolic CAWSS might be explained by time resolution-based artefacts.

VASCULAR MEDICINE

The Vagaries of the Cystic Artery at Laparoscopic Cholecystectomy

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Introduction: There has hopefully been more ink used in the definition and description of the anatomical variation of the cystic artery than blood spilt in the performance of cholecystecomy. However, it is a fact that injury still occurs to biliary anatomy. At its very basic, the cystic artery is a single artery that divides off the right hepatic artery. However, it has been shown that there is a wide array in variation, with approximately 20-25% of the population exhibiting anatomical difference of the cystic artery. This variant anatomy is important to know and understand when identifying structures at cholecystectomy. The aim is to evaluate and appreciate the extent of anatomical variation of the cystic artery, with a view to its impact in laparoscopic cholecystectomy to that already described in the literature.

Material and Methods: The study comprised photographic and written recording of 32 consecutive elective patients undergoing laparoscopic cholecystectomy, over an 8-week project study. Laparoscopic cholecystectomy was carried out using four ports. Exposure of the cystic pedical was achieved using French technique in all cases.

Results: Of the 32 patients, 23 (71.9%) had a single artery, 5 (15.6%) a double artery, 3 (9.3%) cases had three or more arteries. 1 patient the artery was not identified.

•23 cases with single cystic artery: 19 (61.2%) found within Calot's triangle (posterior to duct); 1 of these cases the artery was lying on the duct. 1 case superior to the duct. 2 cases anterior to the duct. 1 case the artery originated from the gallbladder bed and inserted between Hartman's pouch and the fundus of the gallbladder.

•5 cases with double cystic artery: 4 cases had one artery anterior and one posterior to the duct; of these, two cases the posterior artery was closely lying to the duct. 1 case, two arteries were posterior to the duct both within Calot's triangle; this case had a unique spiral artery arrangement, not previously described and different to that of 'cystic artery syndrome'.
•3 cases with triple cystic artery: were arranged with one artery anterior to the duct and two posterior each with different relations to the duct.

Conclusion: Whilst there have been several attempts to classify cystic artery anomalies, due to wide variability, careful dissection of Calot's triangle in every patient is an essential component to identifying aberrant anatomical variation.