

SOCIÉTÉ BELGE DE MÉDECINE INTERNE
BELGISCHE VERENIGING VOOR INWENDIGE GENEESKUNDE

Abstracts sélectionnés comme communications
libres pour le "The Young Investigator's Award"
présentées lors du
congres du 5 Mai 2001
Het Pand, Onderbergen 1, 9000 Gent
Belgique

Abstracts geselecteerd als vrije communicatie
voor "The Young Investigator's Award"
voorgesteld op het
congres van 5 Mei 2001
Het Pand, Onderbergen 1, 9000 Gent
België

FIBRATES AND HOMOCYSTEINE

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Fibrates are used for treating dyslipidemias. Hyperhomocysteinemia is considered as a risk factor for cardiovascular disease. Some data suggest that fibrates increase homocysteine levels. The aim of this study : analyze homocysteine levels in patients treated with these drugs and the effect of vitamin supplementation. 65 patients treated with fibrates (fibrate group) were compared to 64 patients treated with statin (statin group) and to 105 control volunteers (control group). Homocysteine levels were found significantly ($p < 0.001$) higher in the fibrate group than in statin and control groups (16.0 ± 5.2 versus 10.9 ± 3.5 and 10.0 ± 4.6 $\mu\text{mol/l}$ respectively). The difference in homocysteine levels was not explained by differences in age nor in blood levels of B9 and B12 concentrations. Plasma creatinine levels were also significantly ($p < 0.01$) higher in the fibrate group than in statin and in control groups. In 61 patients treated with fibrates, the fibrate was stopped and replaced by a statin during 4 weeks. Then, the patient was rechallenged with the fibrate for another 4 weeks. The homocysteine levels dropped from initial levels of 16.0 ± 5.2 $\mu\text{mol/l}$ to 11.7 ± 2.9 $\mu\text{mol/l}$ with statin and rose again to 17.2 ± 4.8 $\mu\text{mol/l}$ with fibrate rechallenge. Creatinine levels changed in parallel but the changes were not significantly related to those in homocysteine levels. Vitamin supplements (B9, B12, B6) were given for 4 weeks to 47 patients with fibrates : homocysteine levels dropped by 22 %. This lowering effect of vitamin supplements was not statistically different from that found in 29 hyperhomocysteinemic patients not receiving fibrates. The changes in homocysteine levels were correlated only with changes in B9 levels. Conclusion : this study clearly demonstrated that fibrate intake increases homocysteine levels. This increase is reversible, is associated with an increase in creatinine levels and can be partially reversed by vitamin supplementation.

INFLUENCE OF SOURCE, PHENOTYPE AND EX VIVO CULTURE CONDITIONS ON THE HOMING AND EXPANSION OF HUMAN HSC IN THE IN VIVO NOD/SCID MODEL

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Human umbilical cord blood (UCB) hematopoietic stem cells (HSC) have been shown to engraft, differentiate and proliferate in the hematopoietic tissues of sublethally irradiated NOD/SCID mice.

However, very little is known about the homing, lodging and early expansion of human HSC. We used the NOD/SCID assay to study the influence of HSC source, phenotype and culture conditions on these early phenomena in engraftment of human HSC.

We observed that CD34⁺ cells home specifically to bone marrow and spleen during the first 24 hours. Thereafter, the majority of CD34⁺ underwent apoptosis, and by 3 days after injection they were undetectable in all organs except for the bone marrow, the only organ where cell proliferation was observed. In the bone marrow, the CD34⁺ cells proliferated intensively and gave rise to a 12-fold, 5.5-fold and 4-fold expansion in 3 days for UCB, adult mobilized peripheral blood and adult bone marrow derived cells, respectively.

By transplantation of purified subpopulations, it was demonstrated that both CD34⁺38⁻ and CD34⁺38⁺ homed to the bone marrow and expanded, but with different kinetics. CD34⁺38⁺ cells started to increase in cell number from day 3 onwards, and by week 4 after injection virtually all CD34⁺ cells had disappeared and human cells still present were mainly CD19⁺ B cells. In contrast, CD34⁺38⁻ cells remained quiescent during the first week and started to proliferate intensively from the third week on.

Ex vivo expansion of HSC is of clinical interest in the field of transplantation, to improve hematologic recovery or to increase the graft size, esp for UCB, and in the field of gene therapy, to facilitate the transduction of HSC with non-lentiviral retroviruses. The consequences of the ex vivo expansion on the HSC characteristics still remain the subject of controversy.

We investigated the effect of ex vivo culture on the organ selective homing and early expansion in the NOD/SCID model. Cultured cells underwent more apoptosis, had an altered organ selectivity and a changed pattern of expansion and multilineage differentiation, depending on the cytokines used.

We have studied the influence of HSC source, phenotype and culture conditions on the early phenomena in engraftment of human HSC, using the NOD/SCID assay.

Our data provide information of clinical importance, in the field of gene therapy and stem cell transplantation after pathological (HIV) or therapeutic (radiotherapy or chemotherapy) depletion of the hematopoietic system.

IA-2 AUTOANTIBODIES PREDICT RAPID PROGRESSION
TOWARD TYPE 1 DIABETES IN SIBLINGS OF PATIENTS :
PRIMACY OF THE TYPE OVER THE NUMBER OF ANTIBODIES

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Introduction. In relatives of type 1 diabetic patients the risk of the disease has been claimed to increase with the number of different autoantibody types present. We and others have shown that IA-2 antibodies "C" which are often associated with multiple antibody positivity "C" are a marker of impending clinical onset.

Aim. To investigate whether the type or the number of autoantibodies primarily determine progression to diabetes in relatives of type 1 patients.

Methods. Siblings (n=1724; median age [range] : 16 [0-39] years) of type 1 diabetic patients were recruited through the Belgian Diabetes Registry and followed for a median (range) period of 24 (0-131) months. Antibodies against islet cell cytoplasm (ICA) were measured by indirect immunofluorescence and antibodies against glutamate decarboxylase (GADA), IA-2 protein (IA-2A) and insulin (IAA) by radioligand assays. HLA DQ was genotyped in antibody-positive siblings. Kaplan-Meier survival analysis was used to assess progression to diabetes.

Results. On initial sampling 4.2% of siblings were positive for ICA, 5.6% for GADA, 1.7% for IA-2A and 5.5% for IAA. Eleven percent (n=188) presented at least 1 antibody type and 2.0% 3 or more types. Twenty-five siblings (14 males, 11 females) developed diabetes after a median (range) follow-up of 13 (2-77) months. The prevalences of antibody-positivity on initial sampling and later during the preclinical phase were respectively 52 and 76% for ICA, 64 and 68% for GADA, 56 and 72% for IA-2A, 48 and 64% for IAA, 92 and 100% for at least one antibody, and 44 and 68% for at least 3 antibodies. The progression to clinical onset tended to increase with the number of antibodies present, reaching 44% within 4 years for $n_{\geq 3}$ ($P < 0.001$ vs $n_{\leq 1}$); however, there was no significant difference between subjects with 2, 3 or 4 different markers. IA-2A positivity on first sampling was associated with 70% progression to diabetes within 4 years vs 1% in case of IA-2A negativity ($P < 0.001$). In the absence of IA-2A, positivity for 2 or 3 other antibody markers did confer less than 20% risk of diabetes within the next 6 years ($P = 0.0012$ vs IA-2A positivity). In IA-2A positive siblings the progression rate tended to increase further with IA-2A levels ($P = 0.055$ for the highest tertile vs the rest) and the presence of the HLA DQ2/DQ8 high risk genotype ($P = 0.08$ vs non DQ2/DQ8) but not with age or the number of other antibodies present. Eighty-seven percent of the subjects who seroconverted to IA-2A positivity remained IA-2A positive during follow-up.

Conclusions. In siblings of type 1 diabetic patients a single positive IA-2A test rather than multiple antibody positivity per se predicts impending clinical onset. IA-2A levels and HLA DQ status may further refine diabetes prediction. These findings provide objective criteria for a more efficient enrolment of siblings with homogeneously high risk of diabetes into secondary prevention trials. Such strategy is likely to reduce the cost of screening and the number of subjects needed to treat.

CLINICAL SIGNIFICANCE OF ELEVATED SERUM LDH
UPON ADMISSION IN INTERNAL MEDICINE

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Introduction. Serum lactate dehydrogenase (LDH) are routinely measured upon hospital admission in internal medicine (IM). Their elevation (>340 IU/L) is common, has multiple possible severe etiologies, and may face the attending internist with uncertainties.

Methods. Serum LDH were measured upon admission (day 1) in 319 consecutive patients of our IM unit during six months in 1998. The 103 cases (32%) with elevated LDH were further evaluated (mean age 67 yrs ; women 61%). On day 2, the IM resident was asked about her/his explanation for LDH elevation. On day 3, blood tests (haptoglobin, troponin, CPK, SGOT, SGPT, bilirubin) and liver ultrasound were performed. After hospital discharge, the medical record was reviewed (e.g. medical history, final diagnoses) to search for the LDH etiology according to seven pre-defined diagnostic categories : red blood cells (RBC), heart, muscle, liver, thrombo-embolism (DVT-PE), cancer, and unknown.

Results. On day 1, median LDH was 447 IU/L in the 103 patients. On day 2, IM residents suggested that LDH elevation was due to a liver (23%), muscle (14%), cancer (11%), RBC (8%), DVT-EP (7%) or heart (5%) disease, or had no hypothesis (31%). On day 3, LDH had decreased by 12%. After discharge, 56 cases (54%) were still unexplained, while a definite cause was found in 47 cases : cancer (17%), muscle (12%), RBC (7%), DVT-PE (6%), liver (3%) and heart (1%). The resident hypothesis on day 2 had a 64% global true positive rate (i.e. sensitivity) as they correctly identified 30 of the 47 final diagnoses. They had missed only 1 of 6 DVT-PE but 10 of 18 cancer diagnoses. LDH decrease was smaller in the cancer cases (4 vs 15%) on day 3.

Conclusion. Cancer is a main cause of unexplained LDH elevation that should be searched for particularly if high LDH is confirmed. Significance of LDH of unknown origin deserves further studies.

PERSISTENCE OF CHLAMYDIA PNEUMONIAE ANTIGENS
BUT ABSENCE OF SPECIFIC DNA IN ATHEROSCLEROTIC
PLAQUES

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Purpose : To evaluate the presence of various *C. pneumoniae* components in a wide spectrum of atheromatous lesions in different human arteries by applying immunocytochemistry (ICC) with different monoclonal antibodies and polymerase chain reaction (PCR) assays on different target genes, after controlling for the presence of PCR inhibitors.

Methods : 92 atherosclerotic specimens were examined by PCR. For amplification, the primers HL1-HR1 (Campbell et al, 1992) were used. Amplified products were detected by agarose gelelectrophoresis. On a subgroup of 24 specimens an additional PCR was performed with primers 53.1 and 53.2 (Kubota et al, 1996). Analysis of amplified products was done by agarose gelelectrophoresis as well as by hybridization with 2 biotin-labeled DNA probes. A semi-nested PCR protocol was also performed by an independent laboratory in Zürich. For detection of *C. pneumoniae* by ICC, a *C. pneumoniae* specific anti-membrane protein monoclonal antibody, RR-402, a *Chlamydia* genus-specific anti-lipopolysaccharide monoclonal antibody, CF-2 and a *Chlamydia* genus-specific anti-heat shock protein (hsp60) monoclonal antibody were applied. All sections were scored semi-quantitative. To identify macrophages and smooth muscle cells, a monoclonal antibody directed to CD68 and to muscle actin were used. PCR and ICC was also performed on 5 normal aa. mammaaria.

Results : All specimens had moderate to severe disease (Stary grading). PCR assays for *C. pneumoniae* specific DNA were all negative. Cells positive for Chlamydial antigens were found in the same area as cells positive for CD68 and muscle actin. The anti-hsp60 antibody gave 17 %, the anti-LPS antibody 11 % and the RR-402 antibody 79 % of positivity. All tissue sections positive with a genus-specific antibody, were also positive with the species-specific antibody. Semi-quantitative scoring revealed that a higher number of cells was positive when stained with RR-402 antibody. No significant difference was found between detection and type of vascular tissue and between detection and disease stage. All control vessels were classified as Stary grade I lesions. All were negative for *C. pneumoniae* DNA, but ICC staining revealed a high positivity.

Conclusion : We failed to detect *C. pneumoniae* DNA by using three different primer pairs. On the contrary, ICC staining gave high positivity. The most likely explanation is that the DNA is degraded after infection first, followed by Chlamydial LPS and hsp60, and that there is a persistence of membrane proteins. Also, possible presence of Chlamydia-like microorganisms, and therefore cross-reactions, should be taken in consideration.