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Endothelial progenitor cells in patients with splanchnic ischemia: a possible diagnostic marker

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Introduction

The most common causes of splanchnic ischemia (SI), which is a disorder caused by a decreased intestinal blood flow, are atherosclerosis and thrombosis. SI can result in a bowel infarction. The main symptom of SI is abdominal pain. Since abdominal pain is found in many different pathophysiological processes, there is often a delay in diagnosing SI. For this reason SI has a high mortality rate. A diagnostic marker may contribute to shorten the delay in diagnosing SI and is therefore of high value. In this study a possible diagnostic marker for SI has been investigated by flowcytometric analysis: the endothelial progenitor cell (EPC).

EPCs are $CD34^+$ hematopoietic progenitor cells from adults which can differentiate to an endothelial phenotype (1). EPCs comprise a heterogeneous subpopulation of bone marrow mononuclear cells. EPCs express marker molecules which are related to the endothelial cell lineage. These markers give the possibility to isolate EPCs from the total population of mononuclear cells via flowcytometry (2). At sites of ischemia, EPCs are incorporated into newly formed vessels. To rescue tissue from critical ischemia, a possibility would be the improvement of neovascularisation and re-endothelialisation (3).

Studies indicate that bone marrow-derived or circulating blood-derived progenitor cells are useful for therapeutically improving blood supply of ischemic tissue (4, 5). The progenitors ($CD45^{+/-}$, $CD133^+$, $CD34^+$, VEGFR-2(KDR)/$CD14^-$) represent a small population with proliferative potential. They are able to initiate late endothelial outgrowth (6).

Materials and Methods

Patients and Controls

In our measurements we used two different groups; patients with SI (n=13), and a control group with non hospitalized persons (n=10). Patients with SI are subdivided in two groups: patients with coeliac artery compression syndrome (CACS) (n=11) and patients with two-vessel chronic splanchnic syndrome (n=2). EDTA blood was collected and measured within four hours.

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Flowcytometrically determination of EPCs

200 µl EDTA-blood was incubated with the following antibodies: CD45-FITC (20µl) (Beckman-Coulter), CD133-PE (15µl) (MACS Milteny Biotec GmbH), CD34-ECD (20µl) (Beckman-Coulter) and VEGFR2(KDR)-APC(10µl) (R&D systems). During each determination isotype antibodies were used as a control. All tests were performed in duplicate.

After incubation, the antibodies were lysed using the whole blood lysing reagent kit (Beckman-Coulter), and washed with Isotonic (Coulter Isoton II diluent®). The pellets were resuspended in 2 ml Isotonic solution and analysed with the Cytomics FC500 Flowcytometer (Beckman-Coulter). Since EPCs are scarcely present, $1.0*10^6$ cells were analysed in each determination. Cells weakly positive for CD45, positive for CD133, CD34 and VEGFR-2 were selected and defined as EPCs.

Results

EPCs in control group

In healthy control subjects (non hospitalized persons; n=10, age=20-61 years) a reference interval of 22-56/1.10^6cells was found with a SD of 7.44. The coefficient of variation (CV) was 0.22. The healthy control subjects were also measured in duplo to test the reproducibility. For the reproducibility the standard deviation is 7.44 and the coefficient of variation, CV, is 0.19.

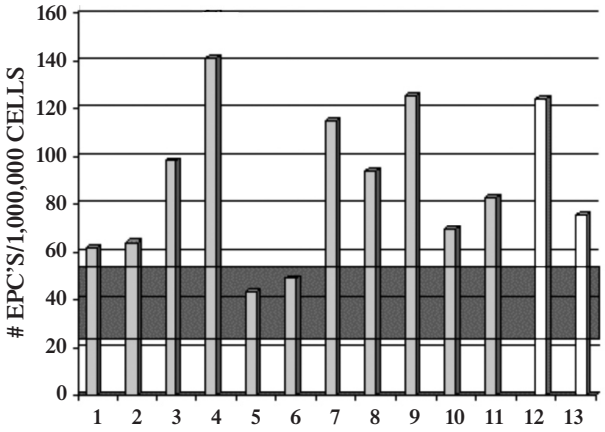

Figure 1. Increased percentages of EPCs were found in patients with one-vessel CSS (▒) and two-vessel CSS (□). The grey area (▒) represents the reference range.

EPCs in patients with SI
Before surgery, 11 out of 13 patients with CSS showed an elevated percentage of EPCs as compared with the control group, as is shown in figure 1. The percentage of EPCs in patients with one-vessel CSS were comparable to the percentage found in patients with a two vessel CSS.

Discussion and Conclusions
It is feasible to determine the percentage of EPCs using the described method. Although the EPCs are present in very low numbers, we found an increased percentage in patients with CSS as compared with the control group. However, we used a small control group to calculate this reference interval and have to increase the number to ensure that this interval is accurate. The coefficient of variation was low, but the reproducibility should also be tested with more controls.
In conclusion, we developed a method to measure EPCs in peripheral blood as a possible marker for CSS. The increased percentages of EPCs in patients with CSS indicate that this marker indeed may be of value for an early diagnosis of CSS. However, more patients and controls have to be analysed to support our hypothesis.

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Evaluatie van de hepatitis-C-test op de cobas e

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Inleiding
De recent uitgebrachte anti-HCV-test voor de cobas e analysers (Roche Diagnostics) is een 'electrochemiluminescence immunoassay' (ECLIA) volgens het sandwichprincipe, geschikt voor het aantonen van antilichamen tegen hepatitis-C-virus (HCV) in humaan serum of plasma. Deze derdegeneratietest maakt gebruik van peptides en recombinantantigenen tegen core-, NS3- en NS4-eiwitten om de anti-HCV-antistoffen aan te tonen. De test is door zijn eenvoud en eenduidigheid van resultaten geschikt voor routinescreening op een klinisch-chemisch laboratorium.

Methode
De nieuwe anti-HCV-methode voor de cobas e werd vergeleken met de huidige HCV-test voor de AxSYM (HCV version 3.0, Abbott Diagnostics), die gebruik maakt van een 'microparticle enzyme immunoassay'-techniek (MEIA). De assay werd uitgevoerd op één E170-machine en één AxSYM, met gebruikmaking van steeds dezelfde meetcel per apparaat. Omdat bij dit vergelijk nauwelijks gebruik gemaakt kon worden van monsters met een bekende titer werd in eerste opzet gekozen voor een vergelijk met de monsters uit de SKML-enquête 'Viral Markers', aangevuld met serologie aan gepoolde sera en patiëntenmonsters.
Vervolgens is een EP10-protocol (1) toegepast op de methode, naar analogie van de initiatie van andere testen op de E170. Vijf opeenvolgende dagen is een serie 'high' (h), 'medium' (m) en 'low' (l) monsters gemeten in de volgorde m-h-l-m-m-l-l-h-h-m. Hieruit is een evaluatie gemaakt van carryover, lineariteit, precisie en bias, gebruikmakend van het programma EP Evaluator (Release 8, David G. Rhoads Associates, Inc.). De uitgangsmonsters waren afkomstig van twee patiënten bekend met hepatitis C. Eén monster had een hoge cut-off-index (COI), het tweede een laag signaal dat verder is verdund met negatief serum tot een waarde rond de cut-off van 1.

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