platelets). Serotonin levels in PPP are increased (142±45 respectively 57±11 nMol/L). Decreased levels of serotonin in platelets might demonstrate diminished synthesis of serotonin due to renal failure.

Conclusion: It is concluded that release of platelet activation factors occur more easily from ?-granulae than from dense granulae.

68. Prevention of amputation by autologous stem cell transplantation in patients with end stage chronic limb ischaemia

R.H. GEELKERKEN, J. SLOMP, T. KAHRAMAN, W. KRUIJER, M. de GROOT, D.W. GRIJPMA, H. WEEKAMP, W. STEENBERGEN, J. FEIJEN, I. VERMES.
Medisch Spectrum Twente, Enschede and the Biomedical Technological Institute of the University of Twente, Enschede, The Netherlands

Introduction: Critical chronic limb ischemia is manifested by pain at rest and nonhealing feet ulcers. Compared with amputation, revascularization is more cost-effective and is associated with better prognosis. Therefore limb preservation should be the goal in patients with critical limb ischemia. We encountered the possibility of vasculogenesis induced by hematopoetic stem cells (1) in patients with progressive ischemic leg ulcers without vascular reconstructive options. The aim of the study was to establish the feasibility and the safety of the treatment.
Methods: Under anesthesia, 800 ml of bone marrow was aspirated from the os ileum. The mononuclear fraction was sorted and the volume was reduced until 60 ml. Cells were injected into the ipsilateral gastrocnemius or soleus muscles on 40 different places. The major clinical endpoints were mortality, complications, amputations and woundhealing. Furthermore, selective angiography to determine vasculogenesis was performed after 1, 6 and 12 months.

Results: Nine patients (7 men, age between 46 and 84 years) were included until now. Six of them were diabetics. The follow-up is between 20 and 1 months. There were no therapy related complications. There were two major amputations necessary. One patient underwent a forefoot amputation for pre-existent necrosis of the toes. Complete woundhealing was observed in 3 patients after 5, 6 and 6 months. Evident vasculogenesis was shown in 1 patient in the 6 and 12 month angiography.
Conclusion: In conclusion, autologous transplantation of hematopoetic stem cells is a feasible and safe treatment in patients with progressive ischemic ulcers of the leg without vascular reconstructive options. Although this is an open study the initial clinical results are promising.

Literature: 1. Tateishi-Yuyama et al. Lancet 2002;360:427-435.

69. Automated result interpretation in anemia testing using artificial neural networks

J. van de VEN, M.G. SCHOORL, P.C.M. BARTELS
Dept. Clinical Chemistry, Haematology and Immunology, Medical Centre Alkmaar, The Netherlands

Introduction: Artificial neural networks (ANNs) can be applied in complicated classification tasks, and have been used in medical decision making. In our laboratory, anemia test results that are ordered by general practitioners are routinely complemented with a human assigned classification code according to the most probable causes of anemia. In this study, we explored the potential of ANNs to learn this particular task.
Methods: We used a standard feed-forward back-propagation ANN model with two hidden layers, available as Excel implementation[1]. Input parameters included: age, sex, ESR, zinc protoporphyrin, Hb, RBC, MCV, MCH, RDW-SD, neutrophilic granulocytes, IRF and reticulocytes (Sysmex XE-2100). Output parameters included: iron deficiency, thalassemia, infection, blood loss, pregnancy and deficient erythropoiesis. Separate ANNs were employed in parallel for each class. ANNs were educated with a training dataset (n=649) using a validation set (n=115) to check for possible overtraining. Candidate ANNs with the lowest training- and validation error rate were selected, and feeded with an evaluation dataset (n=174). For each class, ANNs with the most favorable sensitivity and specificity were evaluated for the acceptability of their output.
Results: Selected ANNs generally had moderate sensitivity (ranging from 50-85%) and good specificity (80-99%). For 21% of anemia classifications, the ANN's output deviated from the human-made classification. With regard to the deviations, 16% (3% of total) were considered critical errors as subjectively judged by an experienced clinical chemist.
Conclusion: The moderate sensitivity and high specificity implicate that ANNs resulting from this particular approach are conservative in detecting an anemia cause, which may be desirable. Resulting errors are mostly acceptable. We conclude that the use of ANNs in anemia classification in a laboratory setting is feasible.

Literature: Saha, A. http://us.geocities.com/adotsaha/NNinExcel.html

70. Verworven thalassemie bij myelodysplasie

A.K. BOER[1], M.C.J.C. LEGDEUR[2], J. SLOMP[1]
Klinisch Chemisch Laboratorium[1], Afdeling Hematologie[2], Medisch Spectrum Twente, Enschede

Inleiding: Afwijkingen in de hemoglobinesynthese zijn over het algemeen erfelijk, maar kunnen ook secundair voorkomen bij hematologische ziekten. Een verworven alfa-thalassemie wordt daarbij meestal waargenomen bij MDS. De reeds opgehelderde mechanismen gaan ofwel uit van een verworven deletie van het alfa-globinegencluster, ofwel van inactiverende somatische mutaties van de 'trans-acting chromatin-associated factor' ATRX. Beide mechanismen resulteren in een ernstig verlaagde expressie van alfa-globine. Ondanks het feit dat onderzoek naar deze deleties complex is, willen we aan de hand van een casus laten zien dat juist eenvoudige bepalingen je op het spoor kunnen zetten van een verworven alfa-thalassemie.
Methode: Vier jaar geleden werd een destijds 69-jarige bakker verwezen naar de internist in verband met ernstige diarree. Bij 'toeval' werd een trombocytopenie geconstateerd. Aanvankelijk werd gedacht aan een milde auto-immuungemedieerde trombocytopenie, maar dit kon niet worden bevestigd. In het beenmerg werden echter wel aanwijzigen gevonden voor myelodysplasie. Aangezien de patiënt geen bijbehorende klachten had, werd deze vervolgd. Twee jaar later werd de patiënt opgenomen met een