

Bridging Continents - Latest Advancements in Haematology

The Sysmex European Haematology Symposium 2009 in Istanbul, Turkey

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In mid-May of 2009 it was time again for the Sysmex European Haematology Symposium which has already been the 5th of its kind in a row. The congress was hosted in Turkey's diverse megacity Istanbul which is a fascinating and colourful mixture of cultures, architectural masterpieces of different epochs, and people from different countries and with different religions. It is not only famous for bridging two continents but also for its role as a capital of many different empires - giving it the nickname *timeless city*. The symposium was held in cooperation with Acibadem University, Istanbul.

More than 470 guests from 49 countries were registered for this symposium - meaning that the number of visitors and the number of home countries is still increasing time

by time! Each time the event is becoming more and more international reflecting its excellent reputation. Soon it might be time to rethink the expression European since every 7th guest came from countries outside Europe! Could you find a better place to host all these people than Istanbul - the city of two continents? Even Napoleon Bonaparte had known about its charm and stated: *If the earth was a single country, Istanbul would be its capital!*

The symposium was opened by **Dr. Rolf Hinzmann**, Director of Medical & Scientific Services, who also chaired the whole congress. He warmly welcomed the guests and led over to the first morning session. As usual, the program of the symposium was diverse and of high quality focussing on up-to-date topics in haematology.



The Blue Mosque is a magnum opus of Ottoman architecture.



Beautiful, elaborate ornaments.

Session 1:

Let's focus on red blood cells: Iron, reticulocytes and NRBCs

Chronic anaemia is an immense public health problem in huge regions of the world, especially in third world countries but also in industry nations. Although the primary cause is iron deficiency, it is rarely present in isolation. The understanding of iron metabolism has undergone a revolution over the past decade. **Dr. Robert Fleming** from the Saint Louis University School of Medicine, USA, gave a lecture on *"Tracing a trace element - An overview of iron metabolism"* and reviewed important pathways. He also focussed on the role of hepcidin, a major key regulator which is vitally important in the pathogenesis of a number of human disorders of iron metabolism, such as iron deficiency anaemia, hereditary haemochromatosis, and the anaemia of chronic diseases.

From the subject of iron metabolism it is only a small step towards the haemoglobinisation of reticulocytes, the precursors of the erythrocytes. **Dr. Herman Ulenkate** from the ZorgSaam Hospital Zeeuws-Vlaanderen in Terneuzen, the Netherlands, implemented the Sysmex parameter RET-H_e for determination of reticulocyte haemoglobin in routine diagnostics and found out that its use increases the efficiency in anaemia diagnostics and can also help saving blood transfusions for orthopaedic surgery. Comparing it to other parameters of iron status like zinc protoporphyrin (ZPP) or haemoglobin, its strong point was particularly the faster response on treatment of iron deficiency anaemia. Whereas a reliable haemoglobin response takes a month, RET-H_e allows drawing conclusions of the success of iron therapy within one week. Furthermore, Dr. Ulenkate showed that the determination of RET-H_e impacts on orthopaedic surgery by enabling patients to avoid transfusions and accelerating recovery.

A very current topic is the use of umbilical cord blood as a source of haematopoietic progenitor cells to replace stem cell transplantation. Cord blood banks have been established in the past years to store the cord blood of neonates for future autologous or allogeneic transplantation of haematopoietic stem cells to treat malignant or non-malignant disorders. Although there is growing interest in this possibility, it is still not very common and well-known. **Dr. Dunja Rogić** from the University Hospital Zagreb, Croatia, gave a presentation on *"Umbilical cord blood - A very special kind of blood and its analysis on the Sysmex XE-2100"* and pointed out towards the necessity to properly determine the number

of nucleated red blood cells (NRBCs) in the cord blood. Only the correct measurement of NRBCs allows the exact numbering of leukocytes which is in turn a prerequisite for adequate estimation of CD34⁺ cells which are measured to estimate haematopoietic progenitor cells. In her study, Dr. Rogić compared different analysers with respect to their ability to correctly enumerate NRBCs and showed that the Sysmex XE-2100 correlated well with the manual count and is therefore useful for the analysis of umbilical cord blood.

Session 2:

African issues: Laboratory logistic loops & iron homeostasis in malaria

Africa, the world's second-largest and second-populous continent, has achieved progress in the improvement of healthcare in certain countries during the past few years. But there are still many countries and regions, especially in sub-Saharan Africa, facing problems which are not even known in developed countries. A big issue in rural parts of sub-Saharan Africa is the access to reliable, timely and accurate laboratory investigations which is a particularly severe problem in areas lacking serviceable roads, electricity supply and landline telecommunication services. **Prof. Barry Mendelow** from Johannesburg, South Africa, talked about *"The African challenge - New technologies to address the laboratory logistic loop in rural health care"* and presented some very unique solutions to the problem. The widespread availability of the GSM networks has contributed to improving communication of processed results from the laboratories to the clinics. Transportation of patient samples from remote clinics to more centralised laboratories is a far more challenging issue. A possible approach to solve this problem is the use of unmanned aircraft systems which have seen extensive military development and deployment in recent years.



Unmanned aircraft systems might close the gap in logistic loops in rural sub-Saharan Africa (Photo by John Robertson).

Apart from the HIV/AIDS pandemic, the African continent is still majorly suffering from malaria, and there is no prospect of an end. According to the WHO, about one million people, 50% of those children below five years, are dying from malaria each year. Malaria is not only the most prevalent tropical disease but also largely contributing to the continued poverty of the continent. Beyond that, malaria induces iron maldistribution and thereby contributes to anaemia. While anaemia is frequently treated with iron supplementation, the therapy bears an additional risk for malaria infected people and can worsen the course of malaria. **Dr. Andre van der Ven** from Radboud University Nijmegen Medical Centre, the Netherlands, investigated the relationship between iron homeostasis and malarial infection in his presentation "*After the mosquito bite - Thrombopoiesis and iron incorporation studies with the XE-5000 Delta-H_e parameter during experimental malaria infection*". He pointed out that iron therapy should not be given during acute malaria and that the Sysmex parameters RET-H_e, Delta-H_e and the biochemical parameter serum hepcidin represent useful biomarkers for selecting children that will benefit from iron supplementation.

Unfortunately, **Dr. Berndt Zur** from Bonn, Germany, was prevented from joining the symposium and presenting his data on the "*Automated WBC differential in CSF and body fluids - Comparative evaluations of the new Sysmex analysers XT-4000i and XE-5000, Siemens Advia 2120 and immune flow cytometry*". Since the manual reference method for CSF analysis is problematical, a reliable automated solution is highly appreciated by many laboratories.

While the audience eagerly awaited some information on body fluid analysis, **Henk Jansen** from Sysmex Europe filled in and introduced the new CellaVision DM1200

system. It is the latest development of digital microscopy devices from CellaVision and can perform slide scans, analyse peripheral blood, and optionally pre-classify blood cells in body fluids.

Session 3:

The Sysmex Outstanding Science Award 2009 - The participants presented their studies to the audience

Sysmex knows and appreciates that many innovative and useful ideas for clinical and scientific studies originate from the customers themselves and may lead to the implementation of new parameters or applications. In order to support this endeavour, Sysmex tendered an award available to all healthcare institutions for outstanding work involving Sysmex technologies in the field of haematology and coagulation. Two years ago, the first award had been given away in Lisbon. Right there, **The Sysmex Outstanding Science Award 2009** had been announced, too. Additional information on the prerequisites of the award was available on the Sysmex Europe homepage thereafter. As in 2007, the feedback on the award was very good and around 40 proposals had been received. The award was well financed with three prizes, the first prize worth € 20,000. The award was supervised by an independent Scientific Expert Committee composed of five distinguished European scientists: Prof. Giuseppe D'Onofrio from Italy, Prof. Andreas Huber from Switzerland, Prof. Sam Machin from UK, Prof. Pranav Sinha from Austria, and Prof. Peter Schuff-Werner from Germany. In March 2008, the Committee met in Munich and first preselected the ten most promising projects which were supported with an initial funding of € 2,000 each. Nine of the ten projects had been finished successfully. Of those, the six participants who did not win one of the first three prizes presented their studies in alphabetical order of the first authors.

Dr. Malte Cremer from the Charité in Berlin, Germany, presented the "*Immature platelet fraction (IPF) as novel laboratory parameter for managing early onset thrombocytopenia in very low birth weight infants*". Thrombocytopenia is seen in up to 30% of all neonates presented to intensive care units. It is associated with an increased risk of intracerebral bleeding. Therefore it is diagnostically valuable to assess the megakaryocytic activity in order to improve management of neonatal thrombocytopenia. The measurement of absolute IPF values may be more suitable than IPF% in case of thrombocytopenia and turned out to be the most efficient approach when combined with the determination of circulating thrombopoietin plasma concentrations.



Interested questions and animated discussions during the symposium.

Dr. Davide Giavarina from Vicenza, Italy, had worked on the "Evaluation of differential white blood cell counting of body fluids using the CellaVision DM96 system". The study considered all kinds of body fluids sent to the laboratory. While cerebrospinal fluid, ascites, pleural fluid, and synovial fluid were all well managed by the DM96, more attention was needed for bronchoalveolar lavage fluid. Taken together, the authors of the study concluded that the DM96 is very useful to standardise the examination between different viewers, but a final review by an expert is necessary.

Dr. Markus Anliker from Austria presented the results of a study using telehaematology applications to connect two haematology centres, the Kantonsspital in Aarau, Switzerland, and the University Hospital of Innsbruck, Austria. "Qualified automated digital imaging device - Useful for telehaematology application?" was the question the project group wanted to answer. For the study, more than 200 blood smear samples were analysed by a CellaVision DM96 device in either Aarau or Innsbruck, and, after sending the electronic data by a network and the physical slides in the manner customary, the respective other institution performed the re-classification. The overall pre-classification accuracy for all cell populations at both sites was good. Re-classification runs led each to comparable medical predications. They concluded: The tested CellaVision DM96 device is suitable for a routine haematology setting. Transport of the slides can be avoided without loss of quality thereby enabling real telehaematology.

The next speaker, **Dr. Micheline Maier-Redelsperger** from Paris, France, worked on a project dealing with "Biomarkers of organic complications in sickle cell disease - Relevance of red blood cell parameters using Sysmex XE-2100". In adult patients with sickle cell disease, chronic organ dysfunction is becoming the major problem and can lead to life-threatening organ failures. In order to identify patients with a high risk of developing those complications, a thorough evaluation of the erythropoietic activity and the haemolytic status is mandatory. Dr. Maier-Redelsperger investigated the usefulness of Sysmex red blood cell parameters for staging of those patients and compared them with standard biochemical markers. The logarithm of RBC-H_e/RET-H_e was found to be the most relevant parameter to investigate haemolysis. Combined with the measurement of lactate dehydrogenase it also provides a marker to predict the risk of proteinuria in sickle cell disease.

The results of the "Validation of a method for automated

schistocyte identification in specimens collected from ART clinics in the Nelson Mandela metropole, with a view to establishing laboratory criteria for early alert to possible thrombotic microangiopathy" were presented by **Mrs. Ahfeyah Agherdien** from Port Elizabeth, South Africa. In the course of the HIV/AIDS pandemic, thrombotic microangiopathy (TMA) and classic thrombotic thrombocytopenic purpura (TTP) have become a more common pathology since both are frequently associated with HIV infection. The study showed that the Sysmex XE-2100 fragmentocyte count is valuable to rule out TMA and TTP, while in parallel the researchers observed a decreased incidence of TMA after the introduction of highly active anti-retroviral therapy (HAART).

Dr. Erwin Kemna from Radboud University Nijmegen Medical Centre, the Netherlands, filled in for **Dr. Edméé van Dongen-Lases**. The study "Diagnosing iron deficiency anaemia in patients with rheumatoid arthritis" aimed to reveal the value of the markers serum hepcidin, RET-H_e, RBC-H_e and Delta-H_e in diagnosing iron deficiency anaemia (IDA) among patients with rheumatoid arthritis. Since it is difficult to diagnose IDA with certainty in those patients, new parameters could be very useful. The results showed that all investigated markers can help to differentiate between the different types of anaemia in the specified patient pool.

Session 4:

The Sysmex Outstanding Science Award 2009 - The lucky winners are ...

The finalists of the award presented their results during the afternoon with the award ceremony and lectures closing and highlighting the first day. The three winners had been chosen by the Scientific Expert Committee prior to the symposium. Something was special about this year's award! As in 2007, three prizes were given away, but in contrast to 2007 there were **two 2nd prizes and one 1st prize** but no third!

One of the two **2nd prizes** worth € 10,000 each went to **Dr. Mauro Buttarello** from Padova in Italy who was honoured for "A clinical evaluation of the %HYPO-H_e and RET-H_e parameters provided by the XE-5000 analyser to predict haemodialysed patients' response to the treatment". In common with the last presentation of the previous session, the study investigated parameters to diagnose iron deficiency but dealt with another patient group. In haemodialysed patients, chronic anaemia is very common due to reduced or prevented erythropoietin production in the kidneys. In the course of treatment with



Dr. Buttarello (left) is happy about the 2nd prize.



Prof. Paltrinieri (second from right) is the other winner of the 2nd prize.

erythropoiesis-stimulating agents (ESAs), iron deficiency might develop if iron supplementation is not adequate. Therefore, a reliable marker of iron responsiveness is needed. The study included 164 healthy adults, 38 subjects with iron deficiency anaemia without chronic kidney disease and 69 haemodialysis (HD) patients, the latter of which were randomly divided into two groups following different guidelines, the European and the American. The haemocytometric measurement of the patient samples was performed on the Sysmex XE-5000. The biochemical parameters like serum ferritin and TSAT% turned out to be poor predictors of iron responsiveness. A higher diagnostic value was demonstrated by using the reticulocyte and erythrocyte parameters. The study allowed concluding that the erythrocyte and reticulocyte parameters %HYPO-H_e and RET-H_e (Sysmex) were more useful than the biochemical markers in managing iron administration and provided information equivalent to the more traditional markers CHr and HYPO% (Siemens). The patients treated by the European guidelines - which mainly differ from the American guidelines by the inclusion of HYPO% - showed an earlier response time to the therapy and needed lower doses of intravenous iron and ESAs.

The other 2nd prize was awarded to **Prof. Saverio Paltrinieri** from Milan, Italy, who was acknowledged with the award for the "*Analysis of canine haematopoietic neoplasms by using the Sysmex XT-2000iV*". The differentiation between reactive and neoplastic proliferation of blood cells can be difficult in dogs; manual review or flow cytometric analyses are often needed. The ability to identify leukaemic cells in veterinary medicine can be increased by using automated analysers like the Sysmex XT-2000iV which can analyse blood samples from several species. User-defined gates can be set and saved for future samples. The study from

Prof. Paltrinieri was performed on 163 canine blood samples classified in four different groups by haematology, bone marrow and lymph node cytology, and immunophenotyping: leukaemic, neoplastic but without blood involvement, non-neoplastic reactive, and healthy. Data were analysed following different approaches (e.g. diagnostic performance of each single parameter or definition of a diagnostic algorithm including all parameters). The Sysmex XT-2000iV turned out to be useful for pre-screening and monitoring of leukaemic dogs, particularly by using additional parameters such as high fluorescence intensity or lysis resistant region events. Moreover, the design of a "leukaemic flag" combined multiple parameters and did not bear the risk to miss out on a leukaemic dog for further diagnostic approaches.

Finally, the winner of the 1st prize was announced: **Dr. Paul Harrison** from United Kingdom was proud to receive it and presented his results on "*Studies on the specificity of the polymethine dye labelling of the immature platelet fraction and its implications for clinical practice*". Measurement of the immature platelet fraction (IPF) is a standardised and precise method providing a valuable non-invasive tool for differentiating between consumptive and aplastic causes of thrombocytopenia, for predicting platelet count recovery, and potentially for avoiding platelet transfusions under certain circumstances. For measurement, the platelets are stained with a fluorescent dye (polymethine and oxazine) that theoretically stains nucleic acids. Similar dyes (e.g. thiazole orange) have been shown to non-specifically label dense granular nucleotides and other membrane systems within the platelet. The study aimed to investigate whether the same is true for the polymethine dye and how the findings might implicate clinical and scientific issues. For the study, 20 apheresis and 20 buffy



Dr. Paul Harrison (in the middle) - the lucky winner of the 1 prize!



The chimera - originally a creature of Greek mythology - possesses a lion head, a goat neck, and the tail of a snake.

coat platelet concentrates were collected and stored under standard blood bank conditions. Samples were then collected on day 1, 3, 5, and 7 and investigated for adhesion and aggregation, thrombin generation ability, platelet activation and microparticle generation, phosphatidylserine exposure, mitochondrial membrane potential, platelet death, and IPF. Unexpectedly, the IPF% and absolute values increased significantly during storage. Taken together, the results of the study suggest that the polymethine dye labelling is presumably not only depending on platelet granules or mRNA. Despite the fact that the labelling mechanism is not fully clear and needs further investigation, the clinical utility of the IPF has been shown in several studies. Recent data also indicate that certain pools of platelet mRNA may be more stable than expected, so the theory behind reticulated platelet or IPF measurement might be different from practice. To fully understand the molecular features of immature platelets and the process of platelet maturation, further investigations will be required.

Session 5:

From chimeras and cases

The morning session on the second day of the symposium started with a very spectacular and provocative question: **Prof. Oskar Haas** from Vienna, Austria, asked "How many am I - how much is me?" and gave "Reflections on the leaky boundaries of individuals and its consequences in biology and medicine". Erroneously, it is common belief that organisms are usually genetically homogenous and that chimeras, individuals possessing cell populations from two or more different fertilisation products, are exceptions and not the rule. Dr. Haas cleared up with this prejudice and explained how chimeras come about, what types of

chimeras exist and what they consist of. The best known example for artificial chimerism is probably intra-species chimerism from bone marrow, stem cell or organ transplantation. Contrarily, natural chimeras usually evolve as a by-product from pregnancy. They derive from as "simple" mechanisms as fetal-maternal reciprocal traffic of cells during gestation, but can also result from as "intriguing" phenomena as germ cell chimerism or whole body/dispermic chimerism. Ultimately, if, for instance, one assumes that a woman usually holds cells of her children and presumably of her mother and her siblings, it seems natural to consider every human being as potentially chimeric.

The following multi-part presentation consisted of three lectures which all covered the central topic "Aiding differential diagnosis with the XE-5000 Case Manager - Sysmex' novel diagnostic concept two years down the line". For a start, **Dr. Frauke Forstreuter** from Sysmex Europe gave an Introduction to the Case Manager and its European multicentre evaluation. With the continuous reduction of healthcare budgets and the simultaneous numerical increase of analytical parameters, the condensation of the relevant information and "interpretative guidance" becomes more and more important. The new Sysmex top-end analyser XE-5000 Case Manager presents a solution to this issue by notifying the user of particular cases when specific sets of haematological parameter combinations are detected. The underlying software works with different algorithms which eventually support a differential diagnosis. The existing cases have been analysed in European laboratories with the appropriate expertise.

Mr. François Mullier from Belgium investigated the circumstances which are "Supporting the diagnosis of hereditary spherocytosis". Hereditary spherocytosis (HS)

is a congenital auto-haemolytic anaemia characterised by the production of sphere-shaped erythrocytes which are more prone to haemolysis than the regular donut-shaped ones. It is the most frequent hereditary haemolytic anaemia in Central Europe with a prevalence of approximately 1:2,500. The study aimed to develop algorithms on the Sysmex XE-5000 Case Manager which are useful to screen for HS, to differentiate the new algorithms from other disorders, and eventually evaluate them. For this reason, a group of 18 HS patients was compared with a group of 105 patients with various haemolytic disorders (e.g. thrombotic thrombocytopenic purpura or haemolytic uraemic syndrome), a group of 93 patients with microcytic anaemia, a group of 57 patients with unknown increased mean corpuscular haemoglobin content (MCHC), and a control group. With those results different algorithms were tested for sensitivity and specificity. One of these algorithms combined more than 10 CBC and RET parameters and lead to an excellent sensitivity of 94.4% and specificity of 99.3% to detect HS.

Subsequently, **Dr. Françoise Schillinger** from Besançon, France, demonstrated the *"Interest of NEUT-X in MDS/MPD diagnosis and chronic monocytosis"*. She investigated the usefulness of the Sysmex parameter NEUT-X in the diagnosis of chronic myelomonocytic leukaemia (CMML). NEUT-X is a parameter reflecting the structure of neutrophils and thus being strongly related to granularity which is significantly lower in myelodysplastic syndromes (MDS) than in anaemic controls. The study revealed that, in the context of an isolated anaemia, a positive granularity index (deduced from NEUT-X) almost rules out the diagnosis of MDS. By means of that, she strongly suggests to include NEUT-X in the routine parameters of Sysmex analysers and to use a decision tree based on the rates of NEUT-X and platelets to diagnose a CMML in the case of a blood monocytosis.

Session 6:

Lessons on leukaemia classification, intensive care management, thalassaemia, and telehaematology

Leukaemia is a very diverse clinical picture; its differential diagnosis can be challenging for many clinicians. With the introduction of new drugs such as imatinib, new diagnostic and follow-up strategies have become necessary as a consequence. **Prof. Torsten Haferlach** from Munich, Germany, is an expert in the field of leukaemia and clarified the latest changes of WHO classification to the audience. In his presentation *"Time has come to change again - The new WHO*



Coffee break around the pool area of the Marmara Istanbul Hotel.

classification of leukaemia" he explained the implementation of old and new information on cytomorphology, histology, immunohistology, immunophenotyping, and more recent methods such as fluorescence in situ hybridisation (FISH) or gene-expression profiling in the diagnostic setting of leukaemias and lymphomas. While Prof. Haferlach explained the complexity of classification, he also advised caution: "Making a diagnosis does not mean to allow a classification!"

With the next talk about inflammatory diseases, another serious and challenging topic was touched. **Dr. Jo Linssen** from Sysmex Europe raised the question *"Intensive care management - Haematological cell analysis scores as a holistic clinical management system for intensive care patients developing inflammatory disease: Imagination or reality?"* in his presentation. It is common knowledge that the diagnosis of inflammatory diseases (ID) is difficult and biochemical markers are rather unspecific and expensive. Although the International Sepsis Conference established a more sophisticated management system in 2003, it seems not suitable for daily routine of most clinicians. In order to improve the current situation, Dr. Linssen investigated the discriminative and prognostic power of haematological cell markers in routine diagnostics of inflammatory disease and concluded that rapid and simple cellular haematology scores could support the treating physician in identifying patients at high risk and defining the course and severity of the disease.

Thalassaemias are considered as congenital haematological disorders which are characterised by a quantitative abnormality of haemoglobin. From Acibadem University Hospital in Istanbul, Turkey, we heard **Dr. Siret Ratip** talking about *"Managing the many facets of thalassaemia - An update on epidemiology,*



Communal drink on Suada island before the dinner starts.

clinics, diagnostics and treatment". He did not only give a very informative overview on history, distribution, types and treatment of thalassaemias but also explained the underlying molecular mechanisms. While heterozygous thalassaemia minor usually does not need any treatment, homozygous thalassaemia major is a very severe disease which causes death within the first couple of life years if untreated. The classical therapy containing regular blood transfusions strongly attenuates the symptoms but also bears an increased risk for acquiring blood-borne infectious diseases like hepatitis B, C, and HIV, especially in countries in which the regulation and control of blood banks is not rigid enough. Future research might lead to the finding of new therapies which could relieve patients to a certain grade from the burden of the disease.

The last scientific presentation of the symposium with the title "*Looking from the far side - CellaVision DM96 and the concept of telehaematology for teaching and diagnosis*" was held by **Dr. Warry van Gelder** from Dordrecht, the Netherlands. He emphasized that the manual blood differential count is still an issue due to its time consumption, the required expertise of the examiner and the interpersonal variance. Moreover, he mentioned that it takes several years to become a professional in morphology. Digital imaging devices have improved immensely in the past years; and digital morphology with the CellaVision DM96 can help maintaining expertise and allows not only the classification of cells but also the use as a teaching instrument. In several surveys, the reproducibility of manual white blood cell classification was tested by using CellaVision's Competency Software. Interestingly, in one survey the same cell was shown twice, and every third examiner did not reproduce the previous classification! Another important advantage of digital morphology is its usefulness for telehaematology applications. By means of that, experts all over the world could take a "look from the far side".

With this presentation, the second day of this interesting and exciting symposium ended. **Dr. Michael Schaefer**, President & CEO of Sysmex Europe, held a short closing speech in which he claimed his impression of the symposium as a wonderful time full of inspiration and ideas. He thanked those who had "delivered the spirit" and reminded everyone that, if we continue with the successful course and concepts, together we can do a lot more than just providing haematological parameters!