Review

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Laboratory hematology in the history of Clinical Chemistry and Laboratory Medicine

Abstract

Background: For the occasion of the 50th anniversary of the journal Clinical Chemistry and Laboratory Medicine (CCLM), an historic overview of papers that the journal has published in the field of laboratory hematology (LH) is presented.

Methods: All past volumes of CCLM were screened for papers on LH and these were categorized. Bibliographic data of these papers were also analyzed.

Results: CCLM published in total 387 LH papers. The absolute number of LH papers published annually showed a significant increase over the years since 1985. Also the share of LH papers demonstrated a steady increase (overall mean 5%, but mean 8% over the past 4 years). The most frequent category was coagulation and fibrinolysis (23.5%). Authors from Germany contributed the most LH papers to the journal (22.7%), followed by the Netherlands and Italy (16.3 and 13.2%, respectively). Recent citation data indicated that other publications cited LH review papers much more frequently than other types of papers.

Conclusions: The history of the journal reflects the emergence and development of laboratory hematology as a separate discipline of laboratory medicine.

Keywords: bibliography; history; laboratory hematology.

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Introduction

Laboratory hematology (LH) arose after the Dutch biologist Jan Swammerdam discovered erythrocytes (red blood cells, RBCs) in 1658, using a primitive microscope that had been invented shortly before by his fellow countryman and colleague scientist Anthonie van Leeuwenhoek [1]. It was not until the second half of the 19th century that hematological investigations were performed on a regular basis in medicine. This became possible after a number of significant achievements had been made, such as the construction of the hemocytometer (counting chamber), improvement of microscopes and Paul Ehrlich’s invention of histochemical staining of blood cells, which eventually led to the Romanowsky stain and its modifications that continue to exist in our times. A real breakthrough in LH was the development of electronic blood cell counting in 1953 by Wallace Coulter [1]. His impedance technology eventually evolved to fully automated hematology analyzers for performing a complete blood count (CBC) that often contains over 40 parameters.

In 1963, when the journal Clinical Chemistry and Laboratory Medicine (CCLM) was founded as Zeitschrift für Klinische Chemie, LH was by no means an existing discipline in what we now call laboratory medicine. Of course, clinical laboratories performed hematological assays routinely, but the attention of laboratory professionals was more focused on technological developments in clinical chemistry than in hematology. It was not unusual to find the hematology work bench in a remote corner of the clinical laboratory. In that period, hematology assays were predominantly manual work using the microscope, although the first instruments became available in the 1960s to the laboratories that could afford them. It was common for a medical technician in mid-sized or larger laboratories to daily analyze a few hundred samples for measuring hemoglobin (Hb): manual pipetting of blood and reagent, mixing, incubating and reading in a simple photometer. The more sophisticated laboratories already had photometers that automatically transformed the measured light absorbance into the Hb concentration. As labor was relatively inexpensive, reimbursement for hematological tests was generally low compared to clinical chemistry assays, in particular when the latter became more and more mechanized and automated. These factors, along
with the slower development of hematology instruments, undoubtedly contributed to the perception that LH had a lower profile than clinical chemistry.

The 50th anniversary of CCLM provides an excellent opportunity for reviewing the development of hematological publications in the journal, as they likely reflect the origin and scientific development of LH as a discipline, and compare it to the other medical laboratory disciplines covered by CCLM and its precursors. Therefore, the present paper reports on the history of laboratory hematology within the framework of CCLM’s history.

### Materials and methods

#### Definitions

As there is no formal definition of what belongs to LH and what not, I have taken the current situation in many European hematology laboratories as the starting point. Included in the definition are subjects like blood cell counting and differentiation, erythrocyte sedimentation rate, cellular analysis in bone marrow and body fluids, coagulation, fibrinolysis, platelet function, blood grouping and blood transfusion. Excluded from the definition are iron metabolism, porphyria, blood gas analysis, glycated Hb, paraproteins, vitamin B₁₂, folate and homocystein. Although the latter items are sometimes closely related with hematological diseases, they are generally not part of the analytical repertoire in hematology laboratories.

#### Search strategy

In order to find relevant publications, I searched all past issues of CCLM and its predecessors: from 1963 to 1966: Zeitschrift für Klinische Chemie, subsequently Zeitschrift für Klinische Chemie und Klinische Biochemie (1967–1975); Journal of Clinical Chemistry and Clinical Biochemistry (1976–1990); European Journal of Clinical Chemistry and Clinical Biochemistry (1991–1997); and finally Clinical Chemistry and Laboratory Medicine (1998–2012). At the time of the search, issue 6 of volume 50 was the latest available (June 2012). The table of contents of each issue was reviewed for publications in the field of laboratory hematology (LH papers), using CCLM’s website (http://www.degruyter.com/view/j/cclm). Types of papers included in the study were original papers, short communications, technical notes, technical reports, reviews, mini reviews, letters to the editor, opinion papers and editorials. Abstracts of congresses and symposia, conference reports and book reviews were disregarded.

#### Categorization

Relevant LH publications were categorized using the Classification terms for submitting manuscripts (http://mc.manuscriptcentral.com/cclm, Attributes subpage), with some refinements where necessary. The classification used is shown in Table 1.

#### Citation data

Citation records of CCLM publications 2009–2011 were retrieved from the ISI Web of Knowledge (http://admin-apps.webofknowledge.com/JCR/JCR), using the classification terms indicated above plus some variations as the search topics. The search was performed on 10 July 2012.
Results

LH papers in the journal

In the 50 years of its existence the journal CCLM published 387 papers on LH subjects. One category was by far most frequently addressed: coagulation and fibrinolysis (91 papers; 23.5%). Then follow papers on instrument and method evaluation (28 papers; 7.2%), platelets (27 papers; 7.0%), pre-analytical phase and hemoglobin (both 22 papers; 5.7%). The categorization of the LH papers is shown in Table 1. The Table also shows the year in which a certain category was used for the first time; this nicely reflects the development of the LH discipline.

During these 50 years CCLM published in total 7669 papers, meaning that papers with LH as the subject constituted on the average 5.0% of all publications. The number of LH papers per year is shown in Figure 1, both in absolute numbers and relative to the number of papers annually published in this period. Figure 1A depicts a steady increase in the number of LH publications, from zero or 1 in the earliest 5 years of the journal to about 20 in the most recent 5 years. Figure 1B makes clear that the share of LH in the journal shows a more or less linear increase, too, from zero in the first years to an average 8% in recent years. The slope of this trend line (1.5% per 10 years) is statistically highly significant (p<0.001).

Major categories of LH papers in chronological order

In the early years, the journal frequently published papers on physiological or physical chemistry studies that were performed on blood cells. The first article in this category was by Pilz, who reported on measuring esterase enzyme activities in serum and RBCs [2]. Because such articles generally contained no relation with clinical hematology applications, this category will not be taken into account in the present overview.

The first paper in the stricter LH category appeared in 1966 and was on the influence of an unidentified small neuraminic acid-rich glycoprotein on the erythrocyte sedimentation rate (ESR) [3]. Interestingly, in their conclusion the authors mention that, “although the ESR for many years belongs to the most frequently performed routine methods, that despite all modern protein differentiations is being used practically unchanged in clinics and private practices, is it important to know that its utility is not only limited by its simple technique, but also by many reactions that continue to occur within 2 h in this ingenious method in a test tube.” It is sobering to realize that this comment could equally well have been written today, albeit possibly in less prosaic language. Not much has changed indeed in the field of ESR over the past 50 years, except the development of new technologies for measuring alternatives to the classical Westergren ESR [4, 5] and controversy about the correct nomenclature of these tests [6, 7].

The first coagulation publication appeared in 1970 [8]. Averdunk and Borner described the results of a study on 77 plasma samples from patients on long-term oral anticoagulants, in which the prothrombin time (PT; Quicktest) was measured using seven different thromboplastin reagents. The PTs showed a highly variable correlation and the authors provided a table for mutually converting Quicktest results obtained with the different reagents. A similar paper, which was more focused on enzyme kinetics of coagulation, was published soon after [9]. Subsequently it lasted over 30 years before CCLM published the next paper on this subject, but then the International Normalized Ratio (INR) was already widely accepted [10, 11]. It proved
unfortunately not possible to trace whether the earlier papers had contributed to the inception of the INR system.

The year 1970 also saw the first publication on a pre-analytical phase subject, although that term had not yet been invented in those days. Hilger and coworkers described blood collection with a disposable micro-capillary for Hb and glucose analysis [12]. The micro-capillary was directly placed into a tube containing the Hb reagent and although a manual assay, the precision was excellent (coefficient of variation <0.8%). Capillary blood has always constituted a problem for hematology analyzers, due to the low sample volume available. But recently a new capillary device was reported, enabling cap piercing and therefore automated sample processing in a hematology analyzer, with reduction of the turnaround time as the main result [13].

Papers on platelets were published in the journal for the first time in 1971, when Schulz et al. described a rapid method for preparing platelet-rich plasma, which was subsequently used in a simple method for counting platelets based on nephelometry [14, 15]. Currently, practically all laboratories use automated hematology analyzers for counting platelets. However, accurate platelet counting continues to be a challenge due to the relatively high prevalence of anticoagulant-induced pseudo-thrombocytopenia [16]. Apart from platelet counting, platelet function has attracted much attention in CCLM also: from a simple platelet adhesion assay using glass beads [17] to aggregometry and sophisticated methods like flow cytometry, electron microscopy and genetic testing [18].

Inevitably, papers on hemoglobin belonged to the earliest LH subjects in CCLM. In 1973 a group from Michigan proposed two micromethods for measuring Hb iron [19] and both correlated very well with the cyanomethemoglobin method that later became the international reference method [20].

The 1975 volume contained two papers on subjects that became pre-eminently characteristic for the journal: one on instrument evaluation and one on quality control [21, 22]. The former was a systematic evaluation of the Technicon Hemalog, at that time the only hematology analyzer that could perform a CBC including platelets [21]. In the same issue these authors reported on computerized quality control of the data produced by the Hemalog, using means of patient results [22]. This was highly innovative in those days; in our times most hematology analyzers offer this possibility as a standard feature.

With regard to cytokines, growth factors and related signal molecules it is not surprising to see that the first publications in the journal were on erythropoietin (EPO): Schulz and Cissé reported in 1976 on their experiments for concentrating EPO in urine [23]. Nowadays, measuring EPO and even thrombopoietin in blood is fairly easy, thanks to the availability of immunoassays and commercial kits for many cytokines [24, 25].

Another characteristic CCLM subject is reference values. As for LH, this subject was covered for the first time in 1980 when Naus and colleagues published their study on deriving hematological reference values from patient data using the Bhattacharya method [26]. Although the validity of this statistical method is not always fully appreciated by some reference values experts, it was recently shown again to be an excellent technique for establishing reference ranges of new hematological parameters [27].

After the introduction of hematology analyzers that could measure CBC components with low imprecision, it became practically feasible to develop the concept of long-term analytical and biological variability in hematology. In 1985 Costongs et al. were the first to present data that proved to be useful for the interpretation of repeat measurements in a single individual, initially for cellular parameters and soon for routine coagulation assays as well [28, 29]. Biological variability data have later been used for defining analytical goals in coagulation testing [30].

In the same period, flow cytometers had reached the stage where they were simple and affordable enough to be used in clinical laboratories. The emergence of the human immunodeficiency virus (HIV) and AIDS in the early 1980s undoubtedly accelerated the development of T-lymphocyte immunophenotyping in clinical routine, as is illustrated by a methodological paper [31]. Not surprisingly, flow cytometric immunophenotyping for the diagnosis of hematological malignancies was later also addressed in the journal [32].

Hemoglobinopathy represents an important field in CCLM. Historically it was a combination of different electrophoresis methods that was used for diagnosing thalassemia and abnormal hemoglobins. In the eighties, high-performance liquid chromatography was introduced in diagnostic laboratories and rapidly became the standard methodology [33]. Today thalassemia screening and diagnosis can no longer be done without molecular techniques [34, 35].

The first paper on measurements on erythrocytes (RBCs) appeared in 1990. The authors observed that approximately 40% of patients on chronic hemodialysis treatment had abnormalities in their RBC volume distribution histograms [36]. One of the possible explanations was obviously iron deficient erythropoiesis. Interestingly, the most recent publication in this category is actually on the same subject: hypochromic RBCs as a marker of iron deficiency [37], demonstrating that it is still a relevant topic. Of recent and rapidly emerging interest is the application
of RBC distribution histogram data as a predictive risk factor for cardiovascular diseases [38].

Closely related to erythropoiesis are of course reticulocytes. The first paper in CCLM was in 1994, when flow cytometric methods had just become available for routine use. Compared to manual reticulocyte counting, flow cytometry was found to be much more precise and enabled improved reticulocyte enumeration of abnormally low counts [39]. A recent review article confirmed the superiority of automated, flow cytometric methods, in particular methods using fluorescent dyes [40]. However, standardization of the reticulocyte count is not yet satisfactory, meaning that instrument-specific reference ranges are still necessary [40].

The history of molecular diagnostics is obviously rather short. CCLM published the first papers on hematological applications in the second half of the nineties: first on a new β-globin mutation [41] and then on a leukemia-specific gene rearrangement [42]. It is especially in the field of leukemia that molecular diagnostics is still in full development and of growing clinical importance [43].

Another major category of LH papers is inflammation, infection and sepsis. The first paper was published in 1999, demonstrating the potential of procalcitonin along with other new biomarkers like cytokines for predicting the outcome of sepsis [44]. Technological advances like flow cytometry of neutrophil CD64 [45] and newer parameters measured in hematology analyzers [46] may help improve the care for patients with this serious and often fatal condition.

Although not relevant for clinical medicine, the abuse of performance-enhancing drugs started to become a niche in LH after athletes were able to obtain recombinant EPO (rEPO) and used it illicitly for doping. Detection of rEPO was the first paper on doping in CCLM, because it constitutes a serious analytical challenge [47]. Since then, the significance of LH for anti-doping work has strongly increased because the athlete’s biological passport is entirely based on hematological parameters. CCLM’s 2011 September issue contained no less than four contributions on this subject [48–51].

The last category to be discussed here is erythroblasts or nucleated red blood cells (NRBCs). For a long time it had been known that the occurrence of NRBCs in peripheral blood was associated with poor prognosis. Yet, new data were presented by Stachon and colleagues, who first described their findings in patients after cardiothoracic surgery in 2001 [52]. By virtue of automated hematology analyzers becoming able to detect and enumerate NRBCs, this field boosted also in CCLM, as evidenced by six more publications, including a very recent one [53].

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of papers</th>
<th>Year of first publication</th>
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<tbody>
<tr>
<td>Germany</td>
<td>88</td>
<td>1966</td>
</tr>
<tr>
<td>Netherlands</td>
<td>63</td>
<td>1975</td>
</tr>
<tr>
<td>Italy</td>
<td>51</td>
<td>1987</td>
</tr>
<tr>
<td>Spain</td>
<td>18</td>
<td>1998</td>
</tr>
<tr>
<td>Belgium</td>
<td>17</td>
<td>1985</td>
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<tr>
<td>Austria</td>
<td>12</td>
<td>1970</td>
</tr>
<tr>
<td>France</td>
<td>11</td>
<td>1989</td>
</tr>
<tr>
<td>Sweden</td>
<td>11</td>
<td>1989</td>
</tr>
<tr>
<td>Finland</td>
<td>9</td>
<td>1988</td>
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<tr>
<td>Poland</td>
<td>9</td>
<td>2002</td>
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<tr>
<td>Slovenia</td>
<td>9</td>
<td>2003</td>
</tr>
<tr>
<td>Croatia</td>
<td>8</td>
<td>1995</td>
</tr>
<tr>
<td>Japan</td>
<td>8</td>
<td>1973</td>
</tr>
<tr>
<td>Australia</td>
<td>7</td>
<td>2003</td>
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<tr>
<td>China (PR)</td>
<td>7</td>
<td>2001</td>
</tr>
<tr>
<td>Switzerland</td>
<td>6</td>
<td>1980</td>
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<tr>
<td>USA</td>
<td>6</td>
<td>1968</td>
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<tr>
<td>Hungary</td>
<td>5</td>
<td>2005</td>
</tr>
<tr>
<td>Korea (ROK)</td>
<td>5</td>
<td>2008</td>
</tr>
</tbody>
</table>

Table 2. LH papers published in CCLM arranged by country of origin (countries with less than 5 LH papers are omitted).

**Authors**

When arranged by country of origin it becomes evident that CCLM most frequently published LH papers from Germany (Table 2). Positions two and three are occupied by the Netherlands and Italy, respectively and at certain distance followed by Spain, Belgium, Austria, France and Sweden. Table 2 also illustrates that CCLM was initially a German-language journal. By the late 1970s, most LH were being published in English. In the next decade more papers were obtained from Western European countries, and in the nineties, scientists from Eastern Europe started to publish LH articles in the journal. Further globalization became apparent in the new millennium, with papers from Asia, Australia and Latin America. However, the number of LH papers from these countries is still limited.

With regards to authorship or co-authorship, my analysis demonstrates that Dr. Jan van Wersch (the Netherlands) has contributed the most LH papers to the journal: 22 over a period of 14 years. His first publication was in 1985 as a co-author of the paper on biological variability [28] and his last was in 1997 on coagulation and fibrinolysis, which was his predominant field of expertise [54]. The second author who published frequently on LH subjects in the journal is Professor Giuseppe Lippi (Italy), who (co-)authored 18 LH papers in 18 years, starting in 1994 with an article on neutrophils [55] and very recently one on pre-analytical phase [56]. Other authors
contributed between one and seven LH publications to CCLM (data not shown).

**Citations**

Data on citations of LH papers in CCLM by other publications were only available from 2009 onwards. Table 3 shows the 10 most cited LH publications. These 10 publications cover eight categories, reflecting that the multidisciplinary nature of CCLM also is true for laboratory hematology. The high citation rate of coagulation articles is likely caused by the dominant position of this category in the journal (see Table 1).

Notably, the seven papers with the highest citation rate are all review papers. The remaining three are an original article, a short communication and an opinion paper. Original contributions actually form the vast majority of LH papers in the journal; therefore it is not clear why reviews are the most cited.

**Discussion**

This historic review of laboratory hematology in CCLM revealed that it was not until the early 1970s that more than an occasional paper on a LH subject was published in the journal. Towards the end of that decade the number of LH papers dropped again, and only since the mid 1980s have LH papers formed a consistent part of all CCLM content. As Figure 1A shows, these years marked the beginning of a significant trend of increasing numbers of LH papers in the journal. Not only did the number of LH papers increase, but also its share relative to the total CCLM publications (see Figure 1B). The only reasonable explanation is that LH has emerged as a separately recognizable discipline within laboratory medicine, which concurs with my personal experience as a laboratory professional: I started my career in exactly the same period. Anyway, it is reassuring to see that the share of LH papers is still growing despite an overall increase in the size of CCLM, meaning that the role of LH as a part of laboratory medicine is continuously being reinforced. This development is further supported by the fact that hematology is an integral part of the syllabus for postgraduate training in clinical chemistry and laboratory medicine in the European Union countries [64]. In some countries hematology is even an officially recognized sub-specialism within the clinical chemistry profession.

When comparing CCLM with other journals in the field of laboratory medicine, one can easily identify some subjects that have traditionally gained more interest in CCLM than in the other journals. Among these are instrument and method evaluations, quality control, pre-analytical phase and reference values. This is not only true for clinical chemistry, but also for laboratory hematology, as Table 1 demonstrates. The prominent share of papers on coagulation, fibrinolysis and platelets (Table 1) is notable. This is likely the consequence of technological progress and clinical developments in hemostasis, thrombosis as well as in anticoagulant therapy.

With regard to the country of origin of LH papers, the leading position of Germany is not surprising, given the fact that CCLM is originally a German journal. The second rank of the Netherlands is surprising, however, at least when one considers that it is a small country with currently only about 100 clinical laboratories. It is tempting to speculate on how this has happened. A possible explanation is that laboratories in the Netherlands are traditionally organized as single, multidisciplinary departments, whereas in many other countries separate laboratories for clinical chemistry and hematology are more common. This might mean that authors are already familiar with CCLM from the clinical chemistry part of their profession and so choose the journal for submitting their LH manuscripts. Alternatively it might be that one or more influential and respected scientists create an informal ‘school’ and transfer their research interests to younger colleagues and collaborators, including their preference of journals for publishing their work. The prominent position of Jan van Wersch as an author of LH papers in CCLM might support this hypothesis. Another possibility is that the Associations of Clinical Chemistry and Laboratory Medicine in the larger European countries do have their own

<table>
<thead>
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<th>Author(s) and reference</th>
<th>Category</th>
<th>Number of citations</th>
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<tbody>
<tr>
<td>Favaloro, Lippi and Franchini [18]</td>
<td>Platelets</td>
<td>20</td>
</tr>
<tr>
<td>Samama and Guinet [57]</td>
<td>Coagulation</td>
<td>14</td>
</tr>
<tr>
<td>Favaloro and Lippi [58]</td>
<td>Quality control</td>
<td>12</td>
</tr>
<tr>
<td>Hoffmann [45]</td>
<td>Infection, inflammation and sepsis</td>
<td>12</td>
</tr>
<tr>
<td>Bacher et al. [43]</td>
<td>Molecular diagnostics</td>
<td>11</td>
</tr>
<tr>
<td>Lippi et al. [59]</td>
<td>Coagulation</td>
<td>9</td>
</tr>
<tr>
<td>Favaloro and Wong [60]</td>
<td>Coagulation</td>
<td>7</td>
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<tr>
<td>de Jonge et al. [61]</td>
<td>Cells in body fluids and bone marrow</td>
<td>7</td>
</tr>
<tr>
<td>Lippi et al. [62]</td>
<td>Erythrocytes</td>
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<tr>
<td>Favaloro et al. [63]</td>
<td>Standardization</td>
<td>6</td>
</tr>
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</table>

Table 3 Most frequently cited LH papers in CCLM from 2009 to 2012.
journals with relatively large distribution, which does not stimulate publishing in international journals like CCLM.

The present report has some limitations that the readers should be aware of. First of all, there is no clear cut definition of LH. Therefore, I used the current practice in the majority of laboratories in Europe. Others might rightfully use another LH definition. Then, the terms used for categorizing the LH papers are not standardized and may be subject to differences in interpretation. As mentioned above, I used CCLM’s classification terms as the basis, but had to make some modifications because it did not cover all LH papers. Finally, categorization is inevitably a subjective process, which is well illustrated by a recent paper entitled “Trueness in the measurement of haemoglobin: consensus or reference method?” [65]. Is this a paper on quality control, on hemoglobin or maybe on standardization? I have chosen the former category, but there may be others who would classify this paper in a different way.

Anyway, despite these limitations, I believe that this overview has demonstrated the emergence and development of laboratory hematology as a discipline in its own right, not only in daily laboratory practice, but also with respect to the scientific status of the field, as is particularly reflected by 50 years of CCLM. If the current trend continues, the share of LH papers in the journal will further increase in the future.

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27. Hoffmann JJ, van den Broek NM, Curvers J. Reference intervals
Johannes (Hans) Hoffmann started his career in clinical chemistry in 1976 as a trainee. Once certified as a specialist he became the head of the hematology laboratory in a large tertiary-care teaching hospital in the Netherlands, where he later was also appointed director of the Department of Clinical Laboratories. In 1992 he obtained his PhD in medical sciences at Leiden University, the Netherlands, on a thesis in the field of fibrinolysis. Since 2008 he has been responsible for scientific affairs in hematology with Abbott Diagnostics in Europe. His scientific work comprises over 90 papers in peer-reviewed journals, mainly focused on general hematology, flow cytometry, coagulation and fibrinolysis. He is also author and co-author of several books on laboratory medicine and hematology. He gave numerous oral and poster presentations in congresses and other scientific events. He acts as a reviewer for various journals, including *Clinical Chemistry and Laboratory Medicine*, where he currently serves his last term as an Editorial Board member. He is a member of several international committees and working groups on standardization in laboratory hematology.