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Hemorheology and vascular diseases: Red cell should rub up to the wall, leucocytes should cope with it

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Greetings . . .

Dear friends, dear colleagues, ladies and gentlemen, good evening . . .

It would be unfair to myself, not to say how happy I am on this evening and very proud as well. But such a feeling is linked to several other reasons. The first is personal of course, enjoying the award, the prize, which has just been given to me. But my enjoyment is also related to the gratification of knowing that friendship has proven faithful and efficient. Therefore I would like to thank very much Professor

Sandro Forconi for all he has done for us and that as a matter of his long-standing friendship. Obviously, nowadays, Sandro appears to be the outstanding person who has succeeded in keeping clinical hemorheology alive and still operating all over the world. The work he has done has been crucially important. Besides, the review *Clinical Hemorheology and Microcirculation* is essential to all of us, given that all published material is immediately spread out on the web . . . We have to keep in mind that determined experiments, willingness and unity, along with such a useful tool, in the form of our Journal, will be the determinant factors for more days of life for clinical hemorheology . . .

Warm thanks also to all my friends in the field . . . The French team of course: Jean François Stoltz and Sylvaine Muller, Claude Le Dévéhat and his group, Jean Frédéric Brun, Jean Luc Wautier and many others . . . and many greetings also to John Stuart in England, Herbert Meiselman in Los Angeles, Oguz Baskurt in Turkey, Albrecht Ehrly in Germany, Gehrt Schmidt-Shönbein in San Diego, Carlota Sandhana and Joan Martin e Silva in Portugal, Amparo Vaya-Montana in Spain . . . I shall stop, knowing that whenever you want to be exhaustive in providing thanks, you forget plenty of persons . . .

And now I'd like to give a lecture on the evolution of clinical hemorheology regarding, at the end, the more prominent role of endothelial cells and leucocytes in vascular diseases, but showing, before, peaks and valleys we were obliged to go through, many dead passages and many errors . . .

High regards to ancestors

It's fascinating to realize how very important persons, the founding fathers, were at the origin of our art: Sir Isaac Newton, Jean Marie Leonard Poiseuille, Robin Fahraeus, studying in 1835 the suspension stability of blood and influence of rouleaux . . . and the first Couette viscosimeter in 1890 . . . I should like to say how we are indebted, in France, to Jean François Merlen (1912–1986) for what he did in the microcirculation, describing functions of the microcirculatory unit. But it was during the last 30 years of the last century that clinical hemorheology developed precisely . . . Special mention to our prophet Al Copley, scientist and painter . . . By the way, every time we organized a congress on clinical hemorheology, we were obliged to set up an exhibition of his paintings with a lot of money to find for transportation, insurance, etc. But he was certainly a nice visionary man who has left us a true definition for the basic foundation of our art: saying in 1951 "the entity of the vessel wall and the blood is an organ named the vessel-blood organ . . ." [1].

Overt and covert diseases (1960–1980)

The first approach of hemorheology is very easy, when considering overt diseases, according to Gordon Lowe, showing a spectacular effect of hemorheology, i.e. increase of plasma and blood viscosity on patients with Waldenström's disease, polycythaemia . . . And it is the same also for poorly deformable cells, easy to find in homozygous drepanocytosis, first shown by Shu Chien and Bränemark, when they used the new "red cell filtration technique" invented by Paul Teitel. But as early as that time (around 1985) some authors observed that sickle cells, dense cells are able to stick to the wall . . . adhesion of cells to the wall . . . a new concept indeed we were to meet again! Such concepts, high blood and plasma viscosities along with bad deformability of erythrocytes, were extended to "covert diseases", i.e. vascular diseases. And so, there followed a fascinating period, a real boost of hemorheology, due, in fact, to pharmaceutical companies, two or three . . . Devices and apparatus were available for filtration, red cell aggregation, the Realm of Contraves, Myrenne . . . At this time a huge quantity of data was produced, along with enthusiastic results. Particularly in cerebral and arterial diseases . . . Concepts such as thyxotropy, described by Leopold Dintenfass and Shu Chien, have been the basic ground for new investigation . . . And the filtration techniques became a worldwide tool in hemorheology . . . So called "fluidizing drugs" were supposed to act on red and white cells and to be prescribed accordingly . . .

Hemodilution developed in cerebro vascular accidents, hearing troubles, distal limb ischaemia, etc. As for epidemiology the Edinburgh survey, the Monica study have also applied plasma viscosity. But some clouds accumulated, casting a shadow on all of that. "More matter with less art" William Shakespeare told us . . .

Many undetermined hemorheological findings

Indeed more and more experiments, then clinical data, have seemed undetermined . . . For example ranges of red cell aggregation appeared to be totally dependent on plasma fibrinogen levels . . . [2]. The filters in blood filtration devices were plugged up with white cells rendering such techniques definitely absurd . . . A group of colleagues, before vanishing into the Ether, moved from hemorheological techniques to simply checking fibrinogen, telling us it was sufficient and useful . . . So new surveys and new congresses appeared based on plasma fibrinogen the risk molecule if located in high tertiles . . . Fibrinogen was significantly higher in Edinburgh than in Toulouse suggestive of North-South gradient . . . However a large study (Fite-Nat) carried out by Ludovic Drouet did not find arguments for a fibrinogen gradient in France, i.e. at the national level . . . Many factors interfere on the level of fibrinogen, explaining a large individual variability. At the patient bedside this measurement is not useful for assessing the individual risk except for high values ($>$7 g/l), which are contra-indicating some elective intravascular interventions such as stenting [3]. And therefore LDL-cholesterol and more recently CRP have come up once again as the only useful markers in arterial diseases . . .

Coulter stopped manufacturing viscosimeters, very early and others followed . . . The drug companies disengaged starting with the biggest ones, laying off their medical co-workers, whom we knew so well . . . Would it announce the death of hemorheology? . . .

Fascinating current rebirth of clinical hemorheology

Many new current concepts have allowed clinical hemorheology to start again. As for vascular disease Pr S. Forconi's concept prevailed: secondary hemorheological disorders following ischemia, along with endothelial dysfunction [4]. At the level of microcirculation a lot of interesting experiments are still emergent regarding fluid leakage and blood flow stealing; hemodilution is still prescribed in Binswanger disease and some other cerebrovascular accidents, in intensive care, before surgery, particularly for critical ischemia in peripheral arterial disease and sometime in aged people.

Particularly in diabetes hemorheology appears important, illustrating the concept of JP Ibsister: the plasma volume contraction, also observed in aged patients [5].

The living wall facing active circulating blood cells

But a more important event has occurred which has developed from 1990 until now: hemorheology moved from the flow to the wall and more precisely to the endothelium layer. A new era opened where wall and cells were linked. Attention was first drawn to the importance of circulating white cells, as a risk factor, a point currently important in cardiology: relation of tobacco, number of circulating leucocytes to myocardium infarction. The first idea was to consider that endothelial cells were "activated" so it was possible to show increases of plasma markers of endothelial activation. For example thrombomodulin appeared increased in arterial diseases of different classes [6]. But more precisely, markers related to adhesion of white cells to the wall were found to be elevated in the course of vascular diseases. In our group we found that hypoxia applied to endothelial cells cultivated in vitro increases P-selectin [7]. Around the year 2000 similar results were shown for all kinds of markers and particularly adhesion molecules in all kinds of vessel alterations and occlusion [8–11]. Even red cells have proven able to stick to the wall under pathological conditions [12]. So a new concept emerged: links between shear stress changes, occurring in vessels diseases, influencing mechanoreceptors of endothelium, then its production of nitric

oxide (NO) and adhesive molecules . . . along with other stimulating factors, such as hypoxia and local inflammation. Important disturbances appeared due to lack of NO, the production of cytokines, von Willebrand factor, chemokines and growth factors, along with a central role for adhesive white cells, mainly granulocytes, and invasive leucocytes, monocytes and mast-cells. The role of specific spots on the wall where shear stress is low or irregular, appeared important, basically in post capillary venulae. But also there are "recirculating zones" in arteries and veins. Therefore arteriosclerosis can be emphasised as a geometrically focal disease, allowing cells to adhere, migrate and carry cytokines and growth factors [13]. The growth of plaques is due to disturbed blood shearing at the wall and leucocytes. And finally an unexpected event occurred: the link between hemorheology and adhesion of white cells to the wall. Hyperviscosity and high propensity of red cells to aggregate enhance adhesion, so hyperviscosity is an actual risk factor in vascular disease. Pr O. Baskurt's experiment dealt with this problem, showing that an increase of red cell aggregation, without any other abnormal parameters is per se able to slow down the shear stress at the wall and then diminished NO production . . . an action only due to the change in velocity profiles [14].

Rollers

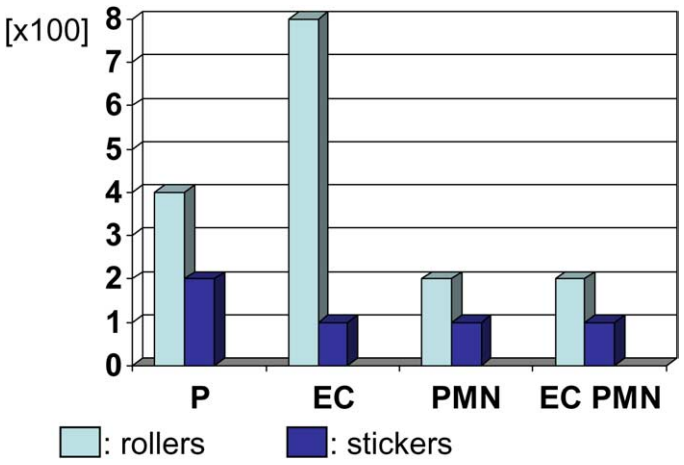
Hemorheology has also encompassed the rolling phenomenon, for example following the impressive experiments of Klaus Ley, from Pr Gaethgens group in Berlin . . . actually in Charlotteville, USA . . . He showed two rollings, fast and slow. Rollings can be separated treating human umbilical vein endothelial cells (HUVEC) with histamine for the expression of P-selectin within seconds, on which rolling is fast or by Interleukin-1 for selectin E (ELAM), on which rolling becomes slow, a prerequisite for the transit of cells. Research, in my group, was performed on rolling, concerning the measurement of action of venoactive drugs on white cells. The idea was to show that a given venoactive drug is able to change the rolling. Using the Gerald Nash device, movements of cells were recorded by video, then, using picture analysing system software, we were able to calculate the distance covered per 3 seconds in a population of human neutrophils. The flavonoid drug was indeed able either to increase the slow rolling, when incubated on endothelial cells, along with a fall in stickers, or to decrease both populations (Table1 [15]). We have thus demonstrated the influence of venoactive drugs on the behaviour of white cells at the surface of the wall, i.e. the recruitment of leucocytes and that this action on rolling is not dangerous, for this is an action on function and not on the available number of circulating cells. This favours efficient action of veno active drugs in chronic venous disease [16].

Chronic vein disease

Another field of interest, for *Clinical Hemorheology* is chronic venous disease (CVD), which is a specific human disorder. When Shrek asks his donkey why he has no risk of varicose veins . . . The answer is that donkeys walk on four legs . . . Indeed, also in the garden of Eden, our ancestor walked on 4 legs and so his ankle pressure was low. But when he gradually acquired the standing position the pressure increased in his legs . . . When he only used two legs the pressure became very high, but he walked all the day looking for food . . . a compensatory effect . . . Alas! It's no longer the same thing nowadays . . . individuals who stand up all the day in modern societies do not walk but drive, have got a very long life expectancy and varicose veins, as a specific human disease, have developed extensively . . .

And hemorheological factors appear to have an important role in CVD, for the high pressure in veins of the lower limbs induces both changes in shear stress and elevated red cell aggregation and hyperviscosity [17]. For example Le Devehat et al pointed out a sensitivity to venous hypertension, obtained by applying a tourniquet to the limbs, i.e. increased fibrinogen, low shear rate hyperviscosity and red cell aggregation [18]. Venous wall remodelling is a direct consequence of that: migrating white cells bearing

Table 1
Change in rolling phenomenon under Influence of a venoactive drug



Numbers of stickers and rollers, mean values, 20 observed fields; P: activation by IL-1 (control); EC: Idem but treatment of the cultured endothelial cells by venoactive drug; PMN: treatment of neutrophils; EC PMN: both cells treated Ref. [15].

growth factor [19]. And so the link is established between hemodynamic factors including hemorheology and tissue biochemistry. The same thing happens with the occurrence of ulcers: such factors are to be found again, with particular role for migration of mastocytes, the carriers of growth factors out of a post capillary venulae. Such cells are able to mobilize TGFβ, then fibroblasts and metallo proteinases and finally the ulcer is generated [19].

For the near future

I am deeply convinced, dear colleagues, ladies and gentlemen, that the future of clinical hemorheology could be productive. For the fields of research in our science are not only the specific physiology of vessels but now is largely open to cell mechanics and biochemistry and genetics as well. All that on the condition that all of us will continue to take up the challenge of hemorheology as an enthusiastic medical science.

All my thanks, once more, to the Council and the Advisory Committee of the European Society for Clinical Haemorheoligy.

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