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Introduction

*A. Pirrelli and G. Cicco***

The existence of a strong relationship between arterial hypertension and hemorheological alterations present in all the vascular segments can be affirmed.

In order to improve the clinical and therapeutic approach to the treatment of arterial hypertension, the research has centred on blood flow in the vessels (this is hemorheology). The main problem has been to study the link between blood flow and peripheral tissue oxygenation. During essential hypertension it is possible to observe changes in endothelial functions and in microvasculature perfusion associated with a reduction in tissues due in part to hemorheological changes such as an increase in blood viscosity or the formation of the blood cell "rouleaux" (following the erythrocyte aggregation that we easily could find during hypertension), which can favour an increase in peripheral resistances and can cause or worsen arterial hypertension.

The arterial hypertension, as known, could be considered as a progressive ischaemic syndrome interesting the macro- and the microcirculation.

Anyway, some aspects are still unclear:

(1) Can arterial hypertension (with its vascular and cardiac remodelling) be the most relevant factor inducing alterations in the macro- and microrheology?

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(2) Can the altered hemorheology, above all in the microcycle, be one of the numerous causes of hypertension?
(3) What is the influence of tissue oxygenation changes in these situations: are these changes causes or effects of microvasculature alterations during hypertension?

We organised this 2nd International Conference in Bari, Italy on 29–31 October 1998 to discuss and collect the main experiences in these fields of research, with the valuable collaboration of Max R. Hardeman Ph.D. and Adrianus J. van der Kleij MD Ph.D. from Academic Medical Center, University of Amsterdam, The Netherlands.

We asked and received the Patronage of

1. Società Italiana dell'Ipertensione Arteriosa	SIIA
2. Società Italiana di Emoreologia Clinica	SIEC
3. Società Italiana per lo Studio della Microcircolazione	SISM
4. International Society on Oxygen Transport to Tissue	ISOTT
5. European Society of Clinical Hemorheology	ESCH
6. International Society of Clinical Hemorheology	ISCH.

At this Conference the Main Researchers in Hypertension, Hemorheology, Microcirculation and Oxygen Transport to Tissue have attended and actively participated as Chairmen and Speakers.

Prof. Enrico Agabiti Rosei	(Univ. Brescia, Italy)
Prof. Gregorio Brevetti	(Univ. Napoli, Italy)
Prof. Gregorio Caimi	(Univ. Palermo, Italy)
Prof. Antonio Capurso	(Univ. Bari, Italy)
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Dr Adrianus J. van der Kleij	(Univ. Amsterdam, The Netherlands)
Dr Vito Vulpis	(Univ. Bari, Italy)
Prof. Alberto Zanchetti	(Univ. Milano, Italy).

Arterial hypertension and hemorheology. What is the relationship?

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The laws controlling hematic flows in the vasculature derive from hydrodynamic laws, but there are some differences:

(1) the arterial tree shows peculiar anatomical and functional characteristics and its segments are coupled both in series and in parallel;
(2) every artery is composed of four segments and the structure of each segment is different and suited to tissue requirements for nutrition, exchanges and physical forces imposed upon the vascular system;
(3) the arterial tree is subject to a variable modeling and remodeling during embryogenesis and throughout the entire life;
(4) blood running through vascular segments has a viscosity attributable both to plasma and to blood cellular components. It can frequently change inducing hemorheological variations and vascular damages.

Arterial hypertension, a complicated disease with vague etiology and with multifactorial pathogenesis, can be considered, in its global and clinical sense, the cause of the major vascular alterations which are the final effect of the transduction of physical or metabolic signals in the vascular tree.

The structure of the various arterial segments differs in the composition of their tissues according to their function, that is, there is a difference in the presence of elastic tissue, the quantity of muscular tissue and the intimal endothelial layer. In arterial hypertension the big arteries may lose elasticity, increase in diameter, increase in wall mass owing to hyperplasia/hypertrophy of the middle muscular layer, and they may become rigid and are often seats of artheromasic and thrombotic alterations.

The earliest clinical sign of hypertension is the impaired compliance of the arteries which can be clinically expressed by the increase in systolic blood pressure without apparent increase in diastolic blood pressure.

The small arteries (2 mm up to 150 μm) mark the transition from the big arteries to the very small ones. They have a fairly thick quantity of smooth musculature.

Their function is still of compliance, but, above all, of resistance to the hematic flow.

During the hypertensive state vessel diameter/lumen ratio increases, and this alteration can be clinically seen in diastolic increase of arterial pressure. The very small arteries (150–8 μm), situated between

the small arteries and the capillaries, are composed only of a thin muscular layer and an endothelial layer. These arteries show an increase in flow resistance, but their function is tied to secretion of endothelial factors (vessel dilators or constrictors, coagulants or anti coagulants, etc.) whose production depends on shear stress and viscosity, but also on alteration in the blood composition (corpuscular and/or chemical components). These arterioles are part of the microcycle together with the capillaries, composed of a single endothelial layer.

The characteristics of microcirculation in hypertension are connected to the phenomenon of the vascular rarefaction. This phenomenon contributes significantly to the increase in resistance at that level and perhaps also to the increase of arterial pressure. This is probably tied to degeneration of endothelial cells or the vascular muscle cells, or rather to an altered angiogenetic mechanism. Most researchers suggest that this continual arterial remodelling is the final result of hemorheological abnormalities induced by hypertension. Blood viscosity and shear stress are phenomena which have a profound effect on the blood flow and on the vascular walls in general. Perhaps this effect is more relevant at the microcycle level.

The microcycle, moreover, is the seat of two opposing phenomena, i.e., "Fahreus-Lindquist" and "Inversion"; the former consists of a reduction in blood viscosity during flow in the microvessels, the latter, on the contrary, of an increase in blood viscosity in the microvessels. These two opposing phenomena could be determined by a different red blood cell viscosity and on their capacity or not to pass through small vessels with a smaller diameter than the red blood cells themselves. The blood composition, partly plasmatic, partly corpuscular, is very important in all the arterial segments, but above all in the microcycle. Increase in rigidity of red blood cells and their reduced capacity to filter, their varying capacity to aggregate and desegregate, different behaviour of membrane dynamic properties, increase in hematocrit values, increase in plasmatic fibrinogen and increase in cholesterol and triglycerides, etc., are often in hypertension. All these alterations have a hemorheological action and greatly influence vascular alterations of arterial hypertension.

New hypotheses lead to the assertion that they themselves may be one of the causes of hypertension.

Conclusions

The existence of a close relationship between arterial hypertension and hemorheological alterations present in all the arterial segments can be affirmed. Some aspects still remain open research and interpretation:

1. Arterial hypertension with its vascular and cardiac remodeling is the most relevant factor inducing alterations in the macro- and microrheology?

2. The altered hemorheology, above all in the microcycle, can be one of the numerous cause of hypertension?

The inflammatory side of hypertension

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The mechanisms that lead to organ injury and arteriosclerosis in hypertension are incompletely understood. In particular, there is a lack of conclusive evidence that has served to establish a link between the elevation of arterial blood pressure and end organ damage in hypertensive patients. In experimental models of hypertension, such as the spontaneously hypertensive rat (SHR) or the salt-dependent hypertensive Dahl rat, and the use of direct *in vivo* techniques, a broad range of microvascular abnormalities has been documented. Besides a shift in blood pressure there are abnormalities in microvascular perfusion pattern,

vascular response to neurogenic or metabolic stimuli, abnormalities in microvascular architecture, lymph flow, and exchange. Furthermore, in the SHR and Dahl hypertensive rat, there exists an immune suppression accompanied by abnormally elevated circulating leukocyte counts in the circulation. The elevated leukocyte count is accompanied by a deficiency in P-selectin expression on microvascular endothelium and incomplete interaction with microvascular endothelium. Since this deficiency can be corrected by adrenalectomy, the evidence suggests a contribution of glucocorticoids to the suppressed immune response. Furthermore, by the use of *in vivo* indicators for oxygen free radicals, an enhanced oxidative stress can be detected in several segments of the microcirculation, including the venules, which is due in major part to xanthine oxidase activity. The oxidative stress predominates in vascular endothelial cells, leukocytes and the plasma compartment. These observations suggest a fundamental defect in vascular oxidative stress which is accompanied by an incomplete inflammatory response due to a deficiency of leukocyte adhesion to vascular endothelium.

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Red blood cell deformability, blood viscosity and tissue oxygenation in hypertension

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Arterial hypertension could be considered a progressive ischaemic syndrome interesting the macro- and the microcirculation. In order to improve the clinical and therapeutic approach to the treatment of arterial hypertenswion, research has centerd on blood flow to evaluate the different components and their very intricate relationships influencing the micro- and the macrocirculation [1–3]. Of course, the main problem is to study the link between the blood flow and the peripheral tissue oxygenation. During hypertension very important alterations in rheological, mechanical and biochemical characteristics of erythrocytes and of blood flow have been shown [4,5]. Very relevant are the increase in blood viscosity, the decrease in red blood cell (RBC) deformability, the formation of RBC "rouleaux" and RBC aggregates. These hemorheological determinants can favour an increase of peripheral resistances and of arterial blood pressure, causing or worsening hypertension, a decrease in oxygen transport to tissue and peripheral perfusion, a decrease of the active exchange surface area in the microvasculature, especially in complicated hypertension [6,7].

We have studied 320 patients: 123 with Essential Hypertension (EH) (M 59; F 64, aged 50 ± 25 years); 81 with Secondary Hypertension (SH) without other associated pathologies influencing hemorheology (M 42; F 39, aged 48 ± 20 years); 116 SH with other associated pathologies or conditions influencing haemorheology such as: diabetes, lipoidoproteinosis, obesity, smoking, HD, elderly, etc. (M 48; F 68, aged 46 ± 20 years). Using a Laser Assisted Optical Rotational Red Cell Analyzer (LORCA) according to Hardeman (1994) we studied Elongation Index (EI) and Angregation Kinetics of red blood cells in these patients. We also evaluated $TcpO_2$, $TcpCO_2$, RPI, $\%Sat.O_2$, ctO_2. In hypertensives we found a decrease in erythrocyte deformability (evaluated with EI), in erytrhocyte aggregation time, a fibrinogenaemia increase, an increase of shear rate to disaggregate erythrocytes, a decrease in cellular oxygen delivery and tissue oxygenation, an impairment of microcirculation. These changes may be involved in the development of arterial hypertension and in its pathogenesis. These patterns also are more impaired in hypertensives with diabetes, lipoidoproteinosis, etc.

These patterns are not related to the age of patients but are significantly and directly ($p < 0.01$) to the patient hypertension-age.

This could be a new way to realize a better treatment in hypertensives and a prevention of cardiovascular complications (i.e.: myocardial infarction, TIA, etc.).

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Red cell fluidity in hypertension

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Common rheological findings in essential hypertension are increases in haematocrit, plasma fibrinogen, plasma viscosity, whole blood apparent viscosity, and erythrocyte aggregation tendency as well as impaired erythrocyte deformability.

Are these abnormalities in hypertension secondary effects of the increased blood pressure via an increased filtration pressure rendering a haemoconcentration or is the initial pressure-increase the result of a deterioration of any of the basal rheologic variables?

Other rheology-associated findings in hypertension are increased levels of intracellular Ca^{2+} reduced plasma volume, decreased insulin sensitivity and increased total peripheral resisstance.

Since the diameter of the red cell is about 8.5 μm, and that of the smallest capillaries about 3 μm, the ability of the cell to undergo deformation is of vital importance for the capillary flow, and a decreased erythrocyte deformability could cause an increased micro-vascular flow resistance.

We have assessed erythrocyte deformability in hypertensive patients by measuring the fluidity of red cells by rotational viscometry and among the findings was a negative correlation ($p < 0.001$) between erythrocyte fluidity and fasting insulin.

Furthemore, during a 2 h euglycaemic insulin clamp the fluidity decreased significantly ($p < 0.001$) in the hypertensive patients.

Associations between increased intracellular Ca^{2+} and decreased erythrocyte deformability on one hand and between *in vitro* insulin and an accumulation of Ca^{2+} in red blood cells on the other have earlier been shown. Hence, there is a possibility that a decreased insulin sensitivity is one important factor in the development of hypertension acting via an impaired erythrocyte deformability and an increased flow resistance in the microcirculation.

Hemorheological disturbances in hypertension. The influence of diabetes and smoking

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It has been recognised for a considerable period that the rheology of blood from hypertensives is disturbed from the normal. However smoking and the presence of diabetes also affect haemorheology. The aim of this study was to investigate the effects of the concomitant presence of hypertension and diabetes, and to compare smokers with non-smokers.

Insulin-dependent (IDD) and non-insulin-dependent diabetics (NIDD) with and without hypertension (HT) were compared with independent age matched controls. The well documented haemorheological alteration from their own control group was observed in both sets of diabetics, in the absence of HT, and could be explained largely in terms of increased fibrinogen levels. When the diabetes was associated with HT in either group the haemorheological disturbances were enhanced still further but still appeared to be largely fibrinogen dependent.

Only the NIDD group was sufficiently large as to allow smokers and non-smokers to be compared. It was found that the known effects of smoking on haemorheology, i.e., resulting from smoking-induced increases in haematocrit and plasma fibrinogen, also occurred in the NIDD's whether hypertensive or not.

Conclusion. The effects of smoking, diabetes and hypertension are additive.

Endothelial function and aging

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The influence of aging on endothelium-dependent and -independent forearm blood flow (FBF) was evaluated through the intrabrachial infusion of endothelial agonists, such as acetylcholine (Ach) or methacholine (Mch), and of endothelial independent vasodilators, such as sodium nitroprusside (NP), both in normotensive (NT) and essential hypertensive patients (HT). In NT aging is strongly inversely related to Ach-induced vasodilation and similar results were obtained with Mch, while the response to NP showed no or only a weak inverse correlation with aging. A similar inverse relationship was found in HT, in whom the vasodilating response to Ach, but not to NP, was significantly reduced as compared to NT along the overall age range. Thus aging impairs endothelium-dependent vasodilation in the forearm of NT and HT. However the effect of aging is a continuous phenomenon starting in young age and continuing up to 70 years. In contrast, NT females appear to be protected against the age-related decline in endothelium-dependent vasodilation as long as they remain normomenstruated. After menopause a steep decline in endothelial function can be observed and in old age a difference between NT men and women is no longer observed. Even in HT patients, gender has an effect on age-related endothelial dysfunction. In HT males, impairment in endothelium-dependent vasodilation is an early event which can be observed in young age (after 18 years). Moreover, up to the age of 45 years, the decline in endothelial function is sharp. After age of 45 years this alteration seems to attenuate. In contrast in HT females, up to the menopause, age-related endothelial dysfunction, although present, is attenuated as compared to males of the same age, while after menopause endothelial function shows a steep decline. In NT endothelial dysfunction is caused by an alteration of the L-arginine–NO pathway starting at the age of 30 years, which can be reversed until the age of 60 years and improved after this age by local infusion of L-arginine. NO availability, as evaluated by local infusion of L-NMMA, is mantained until the age of 60 years, after which it is no longer detectable. This alteration of NO availability is linked to the production of EDCFs and/or oxygen free radicals, as evaluated by local infusion of indomethacin and vitamin C, respectively. In HT L-arginine–NO pathway dysfunction is present until the age of 45 years, and restored until the age of 30 years or improved in HT aged 30–45 years by L-arginine. NO availability is no longer detectable

after the age of 30 years, when EDCF production and oxidative stress appear as the major mechanisms causing endothelial dysfunction. Thus mechanisms causing age-related endothelial dysfunction, i.e., alteration in L-arginine–NO pathway and EDCF-oxygen free radical production, are anticipated in HT.

The VHAS project

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(*on behalf of the VHAS investigators*)

The Verapamil in Hypertension and Atherosclerosis Study (VHAS) is a prospective randomized study with the aim to compare the long term effects of verapamil and chlorthalidone on blood pressure, clinical safety and the progression/regression of carotid wall lesions in a large population of hypertensives. After a 3-week placebo run-in period, 1414 patients with mild to moderate hypertension were assigned randomly to treatment with either 240 mg sustained release verapamil or 25 mg chlothalidone for 2 years. The study design was double blind for the first 6 months and open thereafter. 25–50 mg captopril were added in non responders. In a subset of 498 patients treatment was continued for two further years. In these patients a B mode ultrasound scan was performed at baseline and after 3, 12, 24, 36 and 48 months of therapy to see whether carotid intima-media thickness (IMT) could be influenced by the antihypertensive agents.

Results. After 2 years the systolic and diastolic blood pressure were reduced significantly and to the same extent in members of both treatment groups. The percent of the patients who needed the addition of captopril was 22.6 in the verapamil and 26.2 in the chlorthalidone group. A decrease in heart rate (by 5.8%) as well as a decrease in total serum cholesterol occurred only with verapamil whereas significantly greater rates of hyperuricemia and hypokalemia were observed with chlorthalidone. Among the 456 patients with satisfactory baseline ultrasound readings 33% were classified with normal carotids (SI), 27% with thickenings (SII) and 40% with plaques (SIII). The $M_{\max}$ (mean of IMT at six measured sites) rate of change (mm/year) was significantly smaller in SIII patients (0.003 mm/year) than in SII or SI patients (0.023, 0.025 mm/year). When related to initial $M_{\max}$ values, the rate of $M_{\max}$ change had a negative slope which was significantly different in the two treatment groups (verapamil -0.082, chlorthalidone -0.037 mm/year/mm, $p < 0.02$). Incidence of fatal and non fatal cardiovascular events was greater in SIII patients and among the letter in those randomized to chlorthalidone ($p < 0.05$).

These data show that verapamil has a greater effectiveness than chlorthalidone in inducing regression of thickened carotid artery lesions which is paralleled by a lesser incidence of cardiovascular events rate.

Red blood cell flow properties under oxidative stress

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Red blood cell (RBC) flow properties, namely their aggregability, deformability and adherence to endothelial cells (EC), play a major role in blood flow, particularly in micro-vessels. Oxidative stress (OS) may alter one or more of these properties, and changes in these properties have been linked to microvascular occlusion in numerous pathological states associated with OS. In the present study we monitored the aggregability and adherence of RBC subjected to OS, such as that induced by hydrogen peroxide, phenylhydrazine, photodynamic virucidal treatment, or blood storage, as well as RBC taken from patients with diseases associated with OS, specifically thalassemia major (TM), thalassemia intermedia

(TI), sepsis and acute myocardial infarct (AMI). To distinguish between effects on the RBC themselves and effects on plasma components which might influence these properties (mainly fibrinogen), we compared the aggregability and adhesion of RBC in full plasma to those observed with isolated RBC in buffer. It was found that RBC aggregability and adhesion are differentially altered under these treatments and pathological states. For example: Treatment with H_2O_2 and phenylhydrazine markedly enhanced the adhesion of RBC to EC, but drastically reduced their aggregability. TM RBC exhibit enhanced aggregability and adhesion, while the aggregability of TI RBC is not elevated, but their adherence is markedly greater that of TM RBC. In all these cases the changes in flow properties were observed also with isolated RBC in (plasma-free) buffer, showing that the changes are due to alterations in the RBC membrane itself. In contrast, in sepsis and AMI, enhanced RBC aggregability was observed in plasma, but not in buffer. This was due to elevation of fibrinogen level, as it was diminished by fibrinolytic treatment.

In addition, it was previously shown that OS reduces RBC deformability. OS may alter cell membrane components, such as surface proteoglycans, sialic acid, adhesion molecules, and phospholipid distribution, as well as plasma components. Each of these changes can affect RBC flow properties. Our studies suggest that OS is a diverse phenomenon relating to the presence of a variety of reactive oxygen species and pro-oxidants, which might exert differential and even opposing effects on RBC flow properties.

Hemorheologic alterations in hypertension: chicken or egg?

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An association between elevated arterial blood pressure and abnormal rheological properties of blood appears to be a logical area for investigation, inasmuch as arterial pressure is proportional to the product of cardiac output and total peripheral resistance (TPR). In turn, TPR is determined by vascular geometry (i.e., vascular hindrance) and the resistance to flow of blood: an increased resistance to flow, possibly due to increased blood or plasma viscosity, enhanced RBC aggregation, or elevated RBC or WBC rigidity, would thus be expected to contribute to hypertension.

Chien and co-workers appear to have been among the first to evaluate blood viscosity in hypertension; their results indicate an association between blood pressure and blood viscosity, presumably due to elevated fibrinogen levels and increased hematocrit, and increased viscosity even in borderline hypertension. Subsequent reports have confirmed this association, and recent reviews of hemorheological findings in hypertension have been prepared by London (*Clin. Hemorheol. Microcirc.* **17** (1997), 93–106) and by Ajmani (*Clin. Hemorheol. Microcirc.* **17** (1997), 397–420). Notable findings cited in these reviews include variable increases of hematocrit, elevated plasma and whole blood viscosity, increased fibrinogen and RBC aggregation, and increased RBC rigidity. Our own studies have indicated:

(1) a significant increase in PMN rigidity for hypertensive vs. normotensive diabetic subjects (*Diabetes Care* **17** (1994), 57–63);

(2) a marked effect of both ethnicity and diabetic status on blood pressure, blood and plasma viscosity, fibrinogen and hematocrit (see data by Fisher et al. presented elsewhere at this conference).

Although the current literature clearly indicates hemorheological alterations in hypertension, it fails to fully address two critical questions: (1) are the reported rheological abnormalities the cause of or the result of hypertension?; (2) does therapy designed to restore normal blood pressure also normalize hemorheological parameters? The first question raises the “chicken or egg” problem, and will require extensive genetic and longitudinal studies to resolve. The second has only begun to be investigated (e.g.,

Linde et al., 1996; Mchedlishvili, 1997; Rosenson and Hafner, 1997), and will require extensive clinical trials in order to be resolved. Hemorheology and hypertension may be linked, but the details of this association and its cause-effect relations still remain to be explored!

Relationship of hemorheologic variables with diabetes, hypertension and ethnicity: the Insuline Resistance Atherosclerosis Study (IRAS)

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Background: The IRAS is an epidemiological study designed to assess the cross-sectional relationship between insulin resistance, insulinemia, glycemia and other components of the insulin resistance syndrome (e.g., hypertension, dyslipidemia) and prevalent cardiovascular disease in a multiethnic cohort.

Methods: 1625 White, Hispanic and Black subjects were recruited. Oral and intravenous glucose tolerance tests were performed to establish diabetic status [normal (NGT) or impaired (IGT) glucose tolerance or NIDDM (DIA)] and to evaluate insulin resistance (insulin sensitivity index – SI). Numerous demographic, physical and laboratory measures were examined; those of specific interest here included fasting insulin (IF), plasma viscosity (PV), fibronogen (FIB), white blood cell count (WBC), blood pressure (BP) and waist-to-hip ratio (WHR).

In addition, RBC aggregation (Myrenne) and whole blood viscosity (BV) were measured on a subset of 320 White and Black subjects.

Results: Whites and Hispanics: There were higly significant ($p < 10^{-6}$) stepwise increases in PV, WBC and FIB with diabetic status (NGT to IGT to DIA), and for PV, and WBC the effects of hypertension and diabetic status were additive. PV, WBC and FIB correlated significantly with IF, SI and WHR ($p < 10^{-10}$); these correlations were also significant for the NGT and the normotensive-NGT subgroup when analyzed separately. Blacks: Normotensive-NGT Blacks had higher PV and FIB commpared to Whites and Hispanics. However, in contrast to Whites or Blacks, there was no significant relations between PV and FIB and diabetic or hypertension status. RBC aggregation and blood viscosity showed a similar pattern: In Whites, RBC aggregation and BV at all shear rates were significantly increased in IGT and DIA, and all rheologic measures correlated significantly ($p < 10^{-5}$) with IF and SI. In contrast, in Blacks there were weak or non-existent associations with diabetic status, and the values of all variables in the Black NGT group were similar to those of the White DIA group.

Conclusions: These results demonstrate a close association between hypertension, abnormal hemorheology, and the insulin resistance syndrome.

In particular, the correlation between insulin levels (and SI) and hemorheologic variables, even in healthy non-diabetic and normotensive subjects, suggests a direct role for hyperinsulinemia and insulin resistance in the development of the rheologic abnormalities seen in overt hypertension and diabetes. The results also underline the need to carefully match control groups for hypertension, diabetes and ethnicity in hemorheologic studies, and thus the requirement for caution when attempting to generalize rheologic findings.

Influence of a calcium antagonist on blood rheology and arterial compliance in hypertension

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Beyond their effects on blood pressure, antihypertensive agents may produce additional effects on blood rheology and arterial compliance abnormalities which may play a role in the target organ damage. However these effects may depend only on the specific pharmacological properties of the antihypertensive agents, unrelated to the blood pressure lowering action.

In this study, we compared the effects of nitrendipine (20 mg) to hydrochlorothiazide (25 mg) in 33 mild to moderate hypertensive in a double blind parallel group trial. Blood rheology (fibrinogen, proteins, hematocrit, plasma and whole blood viscosities at shear rate ranging from 0.2 to 128 s^{-1}, red blood cells deformability and aggregation) and radial artery diameter and compliance (Nius 1 – Finapres) were measured at to and t2 months.

Both drugs produced similar blood pressure lowering. Blood viscosity decreased for all shear rates in the nitrendipine-receiving group and increased in the hydrochlorothiazide-treated patients. Red blood cell deformability increased in the nitrendipine but not in the thiazide group. Radial artery diameter and compliance were not different between the two groups but there was a trend to an increase of the cross-sectional compliance in hydrochlorothiazide group and to a decrease in nitrendipine group. The period-group interaction was only marginally non significant.

Our data show that a calcium antagonist (Nitrendipine), but not hydrochloroziade, could improve rheological parameters. On the other hand, the significance and the clinical relevance of radial artery compliance changes may need other investigations.

Evaluation of hemorheological assessment, red cell morphology and intraerythrocytic calcium content in hypertension

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Hypertension represents one of the major risk factor for cardiovascular and cerebrovascular ischemic diseases and the mechanisms through which it can induce vascular damage are both methabolic and mechanical.

The aim of our study was to evaluate hemorheological assessment, considering all its several components (blood viscosity, hematocrit, plasmatic fibrinogen), red cell morphology and intraerythrocytic calcium in two groups of patients both suffering from essential hypertension respectively with or without organ damage. Until now we have studied 20 subjects divided into 2 groups: the former (3 females and 5 males, age between 30 and 76 years) made up by patients suffering from essential hypertension, without recent or previous vascular disease; the latter (3 females and 9 males, age 46–80 years) composed by patients suffering from essential hypertension and cerebral or cardiac ischemia in a chronic phase. In all these subjects we evaluated: blood viscosity by means of plate-cone rheometer by Carri-med (the results obtained were expressed in cPs at a shear rate of 10 s^{-1}; hematocrit using Wintrobe method; plasmatic fibrinogen using coagulative method; red cell morphology according to Zipursky–Forconi method that allows a 3-dimensional observation of red cells suspended in a viscous medium through an optical microscope; intraerythrocytic calcium by means of fluorescent probe FURA2-AM and Perkin–Elmer LS50 spectrofluorimeter (excitation wave at 340 nm for FURA-Ca^{++} and at 380 nm for intracellular free Ca^{++}): the data were elaborated using FL WinLab software and intracellular Ca^{++} values, expressed in nmol/l, were obtained calculating 340/380 (R) ratio in a curve that we have obtained adding digitonin

($R_{\max}$), and EGTA-TRIS ($R_{\min}$), according to the equation [Ca^{++}(i) $= (R - R_{\min})/(R_{\max} - R)$Kd sfb] where Kd $= 225$. Our results show that intraerythrocytic Ca^{++} in patients suffering from hypertension is significantly higher ($p < 0.05$) than in a control group, comparable for sex and age, composed by subjects without hypertension and vascular diseases; (62.09 $\pm$ 10.93 nmol/l in controls, 89.5 $\pm$ 23.11 nmol/l in p. suffering from hypertension, 78.81 $\pm$ 17.76 nmol/l in p. suffering from hypertension with organ damage). In both groups of patients suffering from hypertension that were in wash-out at least from one week, we observed that blood viscosity and fibrinogen were also statistically increased if compared with a control group of healthy subjects. Comparison between these groups leads us to observe that intraerythrocytic calcium is slightly higher in the first group, even if not in a statistically significant way, while blood viscosity and plasmatic fibrinogen are higher in the second group (7.11 $\pm$ 0.58 cPs 10 s^{-1} 1st group, 8.06 $\pm$ 1.32 cPs 10 s^{-1} 2nd group; 306 $\pm$ 46.89 mg% 1st group, 389 $\pm$ 135.94 mg% 2nd group). The study of red cell morphology in all the patients suffering from hypertension pointed out a prevalence in the percentage of discocytes, cells having less deformability, if compared to bowls, with an EMI (Erythrocyte Morphology Index) $= 0.4$; this value decreased in a statistically significant way if compared to controls. Analysing our data we observed that an increase of erythrocytic calcium can be considered an important marker for propensity to hypertension probably due to alterations in the cytoplasmatic membrane permeability with the following cytosolic accumulation of the ion: this condition is also underlined from the increase in the discocytes percentage, which testifies to a reduction in the red cell deformability. Successively, when the illness causes organ damage, the hemorheological alterations, which witness a worsening in microcirculation, are more evident.

Hemorheological and hemodynamic parameters in patients with essential hypertension and their modification by alpha-1 inhibitor drug treatment

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Hemorheological factors play an important role in the pathogenesis of different cardiovascular diseases. The hemorheological and hemodynamic parameters in essential hypertension and their possible modification by antihypertensive treatment were examined in the following two studies.

In the first study the fundus appearance and hemorheological parameters (plasma and whole blood viscosity (WBV), fibrinogen level) of 33 hypertensive patients (mean age: 55 years) were examined.

The fundus appearance showed retinopathy in all the cases between stages I–III. All the measured hemorheological parameters of the examined patients were in the pathological range (WBV at 90 1/s: 5.18 mPa s) and were significantly ($p < 0.01$) higher than in healthy controls (WBV at 90 1/s: 4.18 mPa s). The hemorheological factors showed a parallel deterioration with the fundus appearance, namely their values were significantly ($p < 0.01$) higher in patients with a fundus appearance stage III (WBV at 90 1/s: 6.02 mPa s) than stage I (WBV at 90 1/s: 4.51 mPa s). These results show that there is a correlation between hemorheological parameters and fundus appearance in hypertensives, and this suggests that hemorheological factors may play a role in the development of hypertensive retinopathy.

In the second study the hemorheological and hemodynamical effects of Doxazosin, a selective alpha-1-adrenoreceptor blocker agent, were examined in twenty patients (mean age: 54 years) with essential hypertension. Hemorheologic (hematocrit, fibrinogen, plasma and whole blood viscosity) and hemodynamic (cardiac output and index, total peripheral resistance) parameters and plasma lipids were determined.

The measurements were carried out before the beginning of the treatment, after 1 week and after 12 week treatment periods. Besides significant reduction of blood pressure and total peripheral resistance ($p < 0.001$), a decrease in cholesterol ($p < 0.001$) and triglycerides ($p < 0.01$) levels and a beneficial effect on hemorheological parameters was detected. Fibrinogen and plasma viscosity decreased significantly ($p < 0.01$). Hematocrit value was also lower after one week ($p < 0.001$), then an increase could be seen. Whole blood viscosity showed similar changes as hematocrit, but the degree of its final increase was slighter, which was supported by the significantly lower value of corrected blood viscosity ($p < 0.05$).

All these findings indicate that hemorheological factors may play a role in the pathogenesis and in the development of organ damages in hypertension.

Identification of subjects under risk of ictus recurrence

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Patients (pts) with stroke are at high risk of another stroke. Thus, early identification of patients with such a risk is of clinical importance in order to minimize the risk of another acute event and of its possible dangerous consequences. Identification of the subset of patients at such a risk can be obtained by evaluating 'common' risk factors or in a more accurate way, investigating functional and morphologic alterations of the heart and of great vessel being the possible source of new cardioembolic events. Transesophageal echocardiography (TEE) has an enormous potential in this context. To investigate TEE potential in identifying pts at higher risk of new ischemic stroke after a first stroke the PRIST (prognosis in patients with Recent Ischemic Stroke by Transesophageal echocardiography and carotid echo Doppler) international study was designed and performed. In PRIST study 804 pts with first ischemic stroke were enrolled immediately after the acute event, studied by TEE and carotid ultrasonography and followed up to 1 year.

During follow-up 12.9% of pts had a new stroke, while the remaining (87.1%) did not. PRIST study allowed the identification of subgroups of pts at relatively higher risk of a second stroke. During follow-up, in fact, a new stroke occurred in 31% of pts with left atrial (LA) thrombosis and in 12% of those without; in 24% of pts with LA stasic and in 11% of those without; in 57% of pts with stasic in the left ventricle and in 13% of those without; in 25% of pts with controlateral carotid stenosis and in 12% of those without, TEE and carotid ultrasonography are useful in identifying pts with recent stroke at higher risk of ictus recurrence.

Microcirculation and tissue metabolism in peripheral arterial disease

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Peripheral arterial disease, one of the most common regional manifestations of arteriosclerosis obliterans, is one of the major causes of disability in the middle-aged. The main symptom, intermittent claudication, affects 5% of population over 50 years of age and may profoundly disrupt the patient's day-to-day activities.

Potential causes of reduced capillary perfusion in peripheral arterial disease include abnormal vasomotion, microthrombosis, collapse of capillaries caused by endothelial cell swelling, platelet aggregates,

erythrocytes and leukocytes plugging. These complex disturbances of microcirculation lead to accumulation of acyl-CoAs and other toxic metabolites with consequent impairment in oxidative metabolism and limitation in walking capacity.

Among the drugs utilized to counteract the microcirculatory alterations in peripheral arterial disease, pentoxifyline failed to demonstrate important clinical benefit in controlled clinical trials. Conversely, antiplatelet drugs such as ticlopidine and cilostazol improve walking capacity and ankle pressure in patients with claudication. Arginine, that induces nitric oxide-dependent vasodilation and reduces endothelial expression of cell adhesion molecules, has been shown to increase nutritive capillary blood flow in skeletal muscle of ischemic limb. Improvement in walking capacity and quality of life has been observed in claudicants treated with propionyl-L-carnitine. The precise mode of therapeutic action requires clarification. However, human and animal studies indicate that propionyl-L-carnitine reduces PAF synthesis and free radicals production, increases red blood cell flow velocity, prevents interstitial oedema and improves energy metabolism in the ischemic tissue.

Further studies are warranted to evaluate the efficacy of these treatments in patients with more severe arterial disease, i.e., those with critical limb ischemia.

The precocious structural and functional changes of the cardiovascular system in diabetes-hypertension

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Diabetes is characterized by a high cardiovascular morbidity and mortality and by the frequent occurrence of hypertension. No systematic information is available, however, on how early alterations of the cardiovascular structure and function can be detected. We have recently evaluated wall thickness (T) and distensibility (Dist) in radial artery (RA, -NIUSO2, 10 MHz probe) and in common carotid artery (CA, Wall-Track-System, 7.5 MHz probe). Dist was assessed also in the abdominal aorta (AO) by the same device used for CA (3.5 MHz probe). Blood pressure (BP) was recorded Finapres (RA) and Dinamap (CA and AO) devices and Dist was calculated according to Langewouters (RA) and Reneman (CA and AO) formulae. Cardiac function and structure were echographically studied, either by E/A ratio and H/r ratio. Data were collected in 137 IDDM (age 35 ± 1 ys, age of disease 14 ± 1 ys, HbA1c $8.2 \pm 0.2\%$; means $\pm$ SE) and in 70 healthy controls (C, age 33 ± 1 ys).

In IDDM, BP was significantly higher than in C, while Dist was significantly reduced in all arteries. T was clearly increased in both RA and CA, while, in the heart, E/A was reduced and H/r increased. In IDDM the arterial and cardiac abnormalities were only slightly less evident in absence than in the presence of microvascular complications (retinopathy, nephropathy) and neuropthaty. In hypertensive IDDM, Dist of all arteries was further reduced while T was further increased. The same was observed in the heart where, in presence of hypertension, E/A was further reduced and H/r further increased. Thus IDDM is accompanied by structural and functional alterations of medium and elastic large-sized arteries, and the heart leading to a systemic increase of the arterial stiffness. These changes occur early and may be detected well before the occurrence of microvascular complications. Large artery function and structure abnormalities therefore represent an early vascular dysfunction in IDDM. The occurrence of hypertension has a further negative impact on cardiovascular structure and function.

Study on the optimal treatment for hypertension: introduction to the study

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Cardiovascular diseases are the most common cause of death in Western countries and arterial hypertension is one of the major risk factors regarding both fatal and non-fatal cerebrovascular and coronary complications.

With the introduction of effective, safe and well-tolerated antihypertensive drugs, there has been a substantial reduction in cardiovascular occurrence in the last two to three decades, but notwithstanding this undoubted progress, mortality and morbidity among patients remains higher than that of normotensive cases in the same age bracket. Furthermore, the benefits of the antihypertensive treatment increased as predicted regarding the decrease of cerebrovascular incidence, however the positive impact of the drugs on coronary heart patients was less than expected.

Several hypotheses have been made in the effort to understand these results which are in part surprising. The HOT study to explain two of the numerous hypotheses made. The first regards the diastolic level to be reached with pharmacological treatment in order to obtain the best results. This is often is associated with the controversy brought about by some authors a few years ago regarding the presence of a J or U curve to indicate a pressure reading which corresponds to the most advantageous results, with higher or lower pressures bringing about an increase in cardiovascular incidence.

The other question examined by the study was based on possible benefits, additional to those deriving from the reduction in pressure, even in patients treated with a small dose of a platelet antiaggregant (75 mg/day acetyl-salicylic acid). The relationship between risk factors like arterial hypertension, lipoidoproteinosis, diabetes insipidus, smoking, etc. and arteriosclerosis is commonly measured using prevalence, incidence of cerebral complications (ictus, transient ischaemic attacks) or incidence of cardiac complications (cardial angina, myocardial infarction), which are not truly arteriosclerotic diseases, rather complications resulting from the disease and which can have pathogenic mechanisms which are different from and independent of the risk factor. These cerebrovascular and coronary complications often occur due to the thrombotic occlusion of an arterioscleriotic vessel in correspondence with the rupture or cleavage of the plate.

The antiaggregating platelet treatment has proven to be effective in secondary prevention in patients previously stricken by myocardial infarction and ictus. Primary prevention has not been thoroughly examined or has given dubious or negative results, like the increase in hemorrhegic ictus. On the other hand, the therapy also has some adverse effects, fatal at times. Only a study with the charateristics of the HOT study, that had a large number of patients enlisted and carefully examined in specialised institutes, could face this delicate and important clinical question.

Hypertension optimal treatment: results

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The study involved 18 790 hypertensive patients ranging in age from 50 to 80 (mean age 61.5) in 26 countries, with a diastolic arterial pressure between 100 and 115 mmHg (mean 105 mmHg). The patients were randomly assigned to groups with a target diastolic pressure. 6264 patients were assigned a group

with a target pressure of $\leqslant$90 mmHg, 6264 with a target pressure of $\leqslant$85 mmHg and 6262 with a target pressure of $\leqslant$80 mmHg. Felodipine was administered as the base treatment, and together with other drugs followed a five step regime. Furthermore, 9399 patients received 75 mg/day of acetyl-salicylic acid and 9391 received placebo.

The diastolic arterial pressure was reduced by 20.3 mmHg, 22.3 mmHg and 24.3 mmHg in the three groups with target pressure of $\leqslant$90, $\leqslant$85 and $\leqslant$80 mmHg, respectively. The lowest incidence of major cardiovascular events occurred at an average diastolic pressure of 82.6 mmHg. The lowest risk of cardiovascular mortality occurred at 86.5 mmHg. Pressure readings lower than this proved to be safe. There was a 51% reduction in the occurrence of major cardiovascular events in patients with diabetes mellitus in the target group of $\leqslant$80 mmHg compared to that of the target group $\leqslant$90 mmHg (trend $p = 0.005$).

The acetyl-salicylic acid reduced principal cardiovascular occurrence by 15% ($p = 0.002$) without having any effect on the ictus. Seven mortal hemorrhages occurred in the group treated with acetyl-salicylic acid and eight in the group treated with placebo. There were 129 cases of severe but not fatal bleeding in the first group and 70 in the latter ($p = 0.001$).

The intense lowering of arterial pressure in hypertensive patients was associated with a low incidence of cardiovascular events. The HOT study shows the benefits of decreasing the diastolic pressure to a level of 82.6 mmHg. Acetyl-salicylic acid significantly reduced major cardiovascular occurrence with the best results observed in all myocardial infarction patients. No change was observed in the incidence of ictus and fatal hemorrhages, while cases of severe but not fatal bleeding doubled.

New method for clinical assessment of the microcirculation and tissue oxygenation

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A main problem in the clinical management of intensive care patients is the unavailability of sufficiently sensitive diagnostic bed-side tools to monitor the determinants of tissue oxygenation. The conventionally available global clinical measurements are too insensitive to be able to assess early enough the development of tissue ischemia and organ dysfunction. These early events are important to detect because it is disruption of the microcirculation and oxygen transport to tissue at local levels which precedes the events leading to organ failure. That is why techniques, preferably minimally invasive, need to be developed to assess the presence of local tissue dysoxia (the condition where the oxygen need of the tissue cells exceeds that being supplied by the circulation).

Our working group has been involved in the research and development of optical techniques for the study of pathophysiology of oxygen transport to tissue during ischemia, reperfusion and sepsis. Here the properties of tissue and microcirculation are measured by fluorescence, phosphorescence and reflectance techniques *in vivo* to assess the functional state tissue at the local level.

Oxidative phosphorylation occurring in the mitochondria is the main site for the production of ATP in mammalian cells. Metabolic substrates, ADP and P_i, and O_2 are the ingredients needed to produce ATP. Due to the central role of oxidative phosphorylation in the metabolism of the cell methods directed at measurement of tissue dysoxia will need to measure intermediates of the oxidative phosphorylation in tissue.

One such method enables mapping the distribution of tissue hypoxia by use of a fluorescence technique based on the measurement of mithochondrial NADH as a measure of the cellular energy state. NADH is situated at the high-energy side of the respiratory chain and during tissue hypoxia accumulates

in concentration because less NADH is oxidized to NAD^+. Excitation of NADH by 366 nm light produces, unlike NAD^+, fluorescence at 460 nm light. The NADH signal is considered as a golden standard of tissue dysoxia since the mitochondrial NADH only increases when oxygen need of the tissue cells exceeds that being delivered by the bood in the microcirculation (i.e., dysoxia). We developed an NADH videofluorometer sensitive enough to image NADH fluorescence *in vivo* [1].

Recently a new dye, Pd-porphyrin compound has been introduced by Wilson and co-workers with which it is possible, using quenching of phosphorescence techniques, to quantitatively determine oxygen concentrations in the microcirculation *in vivo* [2]. Use of this techique, although not yet applied clinically, has given us new insights into oxygen transport pathways in shock and inflammation [3] and allowed us to demonstrate the ability of Hb solution to directly improve microcirculatory oxygenation in pigs following hemorrhagic shock [4].

The microcirculation is the physiological compartment responsible for tissue oxygenation and nutricion. It forms a particularly sensitive compartment and is considered to be of paramount importance in the etiology and development of many diseases. Till now study of the of the human microcirculation has been limited to observation by use of capillaroscopic microscopes of the microcirculation of the naifold and the bulbar conjunctiva.We have recently clinically applied a new type device for observation of the microcirculation based on reflectance videophotometry [5]. With this device, which we developed for use in surgery, we were successful, and first, in observing the microcirculation in the human brain. Currently many different clinical uses of this device are being explored [6]. It is expected that this device will become an important clinical device for assessing the functional state of the human microcirculation in disease.

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Microvascular pO_2 in models of shock

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Quantitative measurement of oxygen concentrations in the microvasculature is of prime importance to issues related to oxygen transport to tissue. The introduction of the quenching of the Pd-porphyrin phosphorescence as oxygen sensor *in vivo* has provided in this context a major advance in this area of research. This presentation will give an overview on the calibration, validation and application of this technique in hemorrhagic and septic shock in pigs.

In a study in pigs we investigated the relation between microvascular and venous oxygen pressures during hemorrhagic shock. It showed that during baseline the mesenteric venous pO_2 (60 ± 9 mmHg) and the microvascular pO_2 (60 ± 11 mmHg) had similar values. During shock, microvascular pO_2 dropped significantly below Mes. Ven. pO_2 values (26 ± 10 mmHg versus 35 ± 8 mmHg).

Septic shock was induced by a continous low dose of LPS, until the mean arterial pressure dropped by 25%, which resulted in similar cardiac output and blood pressure as in the hemorrhagic shock group. In this study the microvascular pO_2 dropped again to levels much lower than the venous pO_2 levels (intestine 29 ± 7 mmHg and 45 ± 3 mmHg). However, the divergence between the microvascular and mesenteric venous pO_2 was much larger in the septic group.

The results of these studies are consistent with the notion that oxygen transport to the microcirculation is shunted during shock, resulting in microvascular pO_2 values becoming lower than venous pO_2 values. Shunting of oxygen from arterioles to venules is put forward as an explanation for this observation. Probably diffusive shunting plays a major role in hemorrhagic shock whereas convective shunting seems more important in septic shock.

Fractal temporal patterns in tissue oxygenation in the brain cortex

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Introduction

Red blood cells (RBCs) are the main source of oxygen to the tissue. Both regional RBC-flux and content show spontaneous fluctuations, as seen in our studies [1,2], which can be fully or partly ascribed to vasomotion along the vascular tree supplying the region.

Aim of the study

The fundamental question tested in these studies was whether or not the oxygen supply to the brain cortex can be regarded an orderly process or is it subject to random variations with no characteristic pattern in time. In other words, is it stable, periodically changing or is it more complex than these simple forms?

Methods

Power spectral density (PSD) and fractal analyses were used to describe the frequency spectrum and the degree of temporal correlation between discrete samples of these signals. RBC-parameters were acquired from the brain cortex non-invasively in rat and human studies.

Rat studies. In 5 experiments, RBC-flux was continuously measured by a laser-Doppler flowmeter (Moor MFB3D) in the parietal brain cortex of anesthetized rats through the closed calvarium. Perfusion to the brain was adjusted non-invasively by setting the mean arterial blood pressure to 100 mmHg for a period of 2 minutes by lower body pooling of blood by a computer controlled suction device. RBC-flux time series of 2^{14} elements were created by sampling the signal output at 200 Hz and 12 bits. The Hurst coefficient, H, which characterizes the fractal correlation structure in the data sets was estimated by the bridge detrended scaled windowed variance method (bdSWV) [3].

Human studies. In 5 experiments, total, reduced and oxygenated hemoglobin contents were continuously measured in male volunteers of 21 to 28 years of age by near infrared spectroscopy (Hamamatsu NIRO 500) over the frontal region by placing the optodes underneath the hairline at a distance of 4 cm. Subjects were seated in an armchair and allowed to read at their leisure. Hb-time series of 2^{15} elements were created by sampling the signal output at 2 Hz and 12 bits corresponding to a record of 285 minutes.

PSD plots were used to characterize the power distribution among the frequency components in these signals. The Hurst coefficient was estimated by the bd SWV method.

Results

In both species the temporal record of RBC-flux and Hb content, hence oxygen supply to the brain cortex, exhibited complex behaviour in time. No single frequency (oscillation) but a continuum of frequencies with exponentially declining power towards the high end of the spectrum was identified in the PSD-plots typical of a fractal signal with the following estimates of H: for RBC-flux in the rat 0.29 ± 0.04, for Hb-content in the man 0.16 ± 0.06 (total), 0.22 ± 0.07 (oxygenated). Our findings indicate, that oxygen supply to the brain cortex may look disorderly but in fact in time it folds out according the geometrical order of fractals.

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Membrane fluidity and oxygen diffusion in enriched-erytrocyte membrane

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In contrast to the traditional hypothesis that only the obstructive atherosclerotic plaques progress to ischemia, we investigate a rheological pathway towards ischemia. In the present study, we measured the influence of cholosterol rigidification on the oxygen permeability in membranes of human endhothelial cell monolayers (Ecs).

The cholesterol induced membrane rigidification was verified at different depths of the membranes by a fluorescence polarization method with diphenylhexatriene (DPH) and 1-(4-trimethylamino)-6-phenylhexatriene (TMA-DPH). The fluorescence quenching by oxygen was probed in preferentially labeled membrane with pyrene butyric acid (PyC_4) and pyrene dodecanoic acid (PyC_{12}), as shown with a 3D fluorescence microscopy CellScan System. For both probes, these experiments revealed decreased oxygen diffusion as the cholesterol concentration increased in the medium culture (3.32, 6.84 and 17.11 μM). The oxygen quenching of the long chain PyC_{12} appeared much more dependent on the cholesterol levels than the oxygen quenching of PyC_4. We showed that cholesterol affects particularly the oxygen levels or diffusion rate in the middle region of the membrane.

In conclusion, these findings prove in a direct way that physiological concentrations of cholesterol significantly affect the endothelial barrier function and molecular oxygen transfer process to underlying tissues. Risk factors (cholesterol) directly contribute ischemia [1,2].

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Invasive and non-invasive pO_2 measurements in clinical practice

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Different methods have developed to assess the partial pressure of oxygen in tissue. Some are designed for *in vitro* application or for physiological evaluation, whereas others can be used in clinical practice. In general the degree of invasiveness influences the clinical acceptance. However basic knowledge of microcirculatory regulation mechanisms is required for a good interpretation of the final results.

From a clinical point of view a pO_2 measuring device must fulfil several minimal conditions to be accepted by the physician and the patient. Minimal invasiveness for the patient, easy handling of the device, reliability, immediate result after the measurement, a large enough space resolution and a small temporal resolution are the most important factors. The classical methodology to measure tissue pO_2 is based on electrochemical reduction (polarography) and build into a macro- or microneedle. These types of devices are most frequently used in clinical situations and some investigators still consider this method as the golden standard. For example, in tumour research the polarographic pO_2 needle is used to assess tumour hypoxia in order to predict metastasis [1]. However, methodological limitations must be considered to obtain reliable results [2].

Transcutaneous polarographic pO_2 electrodes were initially developed for non-invasive monitoring of arterial pO_2 in new-borns [3] and are now widely used in clinical practice for numerous purpouses.

Optical non-invasive measurements of oxygen in the microvasculature have been developed during the last 10 years [4]. This methodology is based on two different optical reactions: absorbance and fluorescence/phosphorescence. Fluorescence lifetime imaging of the skin pO_2 has become available for clinical application [5]. Based on oxygen quenching of the fluorescence of an excited fluorophor immobilised in a polymer at the end of an optical fibre assessment of tumour oxygenation has become feasible [6].

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Microvascular changes during laboratory stimuli and structural hemodynamic indices

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Hypertension, since the very early stages, is characterized by hemodynamic disorders and secondary functional and structural damages. Together they compose the 'hypertensive syndrome' that has been related to an enhanced sympathetic drive primarily originated by the Central Nervous System.

Moreover, metabolic abnormalities and exaggerated cardiovascular responses to neurogenic stimuli are common features in borderline-to-mild hypertensives and in normotensives with a risk for hypertension. Then, several authors have suggested that an impaired peripheral blood flow might represent the linchpin between the sympathetic overdrive and the hypertensive syndrome. Nevertheless, very few studies have been performed to demonstrate the relationship between the hemodynamic reactivity and the vascular structural and metabolic abnormalities in hypertensives.

We adopted electrocardiographic impedance, plethysmography and laser-Doppler flowmetry to investigate the systemic and regional hemodynamic changes during psychophysiological stimuli and the effects of macro- and microvascular damage in hypertensives with and without metabolic disorders. In particular, studies regarding the tissue oxygenation and the hemodynamic stress response as psychophysiological markers of hypertension, and the consequences of structural vascular damage on the functional hemodynamic changes will be described. Moreover, findings on the vascular reactivity in hypertensives with insulin resistance and hypercholesterolemia will be presented.

The overall results suggest that sympathetic overdrive, endothelial damage and metabolic abnormalities reduce the peripheral blood flow. Then, they potentate, in a vicious circle, the hypertensive disease and the functional and structural systemic and microvascular disorders.

Modelisation of leukocyte adhesion on a fibrinogen coated surface in static conditions

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Introduction. Leukocyte adhesion plays an important role in many physiopathological phenomena. Polymorphonuclear cells (PMNs) adhesion to vascular endothelium occurs during different vascular pathologies (atherosclerosis, inflammation, leukocyte adhesion deficiency...) and autoimmune diseases (lupus, ...). The knowledge of adhesion processes of cells in different physiological conditions is of prime necessity to understand this phenomenon. A modelisation of adhesion allows the comprehension of this complex process.

Aim of the study. The aim of this work has been to study PMNs interaction with a fibrinogen coated surface, a specific biological substrate [1].

Materials and methods. Adhesion of quiescent of formyl-methionine-leucine-phenylalanine (FMLP) 10^{-7} M activated PMN has been studied with a parallel chamber after sedimentation on a glass slide coated with fibrinogen (0.01% w/v). The activation state of PMNs was both quantified by flow cytometry and controlled by fluorescence microscopy, which allows a 3D determination (Cell-scan, Scanalitics, USA). Influence of the cellular concentration was studied on the cell adhesion deposit process (3, 5,

7×10^5 cells/ml). 200 pictures were taken for each experiment and analysed by an image processing software (Visiolog, Noesis, France) to determine statistical data: homogeneity of cell deposit, radial distribution function $g(r)$ and deduced variance $(\sigma^2/\langle n \rangle)^2$.

Results. The results suggest that quiescent neutrophils deposition could be interpreted as a ballistic deposition. This random adsorption model differs from random sequential theory (RSA) in the way that cells arriving at the surface are able to roll along cells previously adhered and also, for large particles, a strong gravitational force exists.

Conclusion. The deposition process of PMNs has been defined in static conditions. In these conditions, activated PMNs seem to follow a different deposition process compared to quiescent PMNs. More investigations with activated cells and under flow conditions ($0 < t < 3$ Pa) are now necessary for better knowledge of this phenomenon.

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The arteriolar structural changes and their evolution in hypertension

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Structural changes of small resistance arteries significantly contribute to the increased vascular resistance in hypertension, and therefore have probably a significant role in the maintenance of high blood pressure values.

Studies in animal models of genetic or experimental hypertension have suggested that structural changes may occur in the early stage of hypertension, in response to mild elevation of blood pressure or as a primary abnormality. An early treatment with ACE inhibitors or dihydropyridinic calcium antagonists in young SHR may prevent cardiovascular hypertrophy and, at the same time, may reduce blood pressure long-term, even after treatment withdrawal.

Studies in man for the evaluation of arteriolar structural changes have previously rested on hemodynamic measurement of minimal vascular resistance calculated from arterial pressure and maximal blood flow in suitable vascular beds. Forearm Minimal Vascular Resistance can be reduced with the use of different antihypertensive drugs, in particular with calcium antagonists and ACE inhibitors.

More recently, a direct evaluation of vascular structure *in vitro ex vivo* has allowed to detect an almost complete normalization of structural abnormalities after a prolonged treatment with different ACE inhibitors (perindopril, cilazapril) but not with the β-blocker atenolol.

The prognostic impact of the regression of structural alterations in small resistance vessels is presently unknown.

Endothelin, microcirculation and hemorheology

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Although a large amount of data concerning microcirculation and cardiovascular diseases is available, little is known about microcirculation and hypertension. The reasons explaining the very limited knowledge are mostly related to the difficulty of examining selectively capillaries and metaarterioles, independently from small arteries or larger vessels.

Inded, the physiological role of capillaries and metaarterioles, the only two elements which constitute microcycle, is peculiar and tightly committed to metabolic exchanges.

During the hypertensive state, several factors including an elevated plasma viscosity, or abnormal membrane properties of red blood cells, or increased levels of fibrinogen, LDL, hematocrit, can alter these mechanisms. Whether an abnormal release of endothelium derived vasoactive factors from capillaries, or an abnormal production of chemical factors by blood cells running through this district of the vasculature, exist in hypertensives is an intriguing hypothesis but unfortunately, to our knowledge, neither experimental nor clinical data in the literature have demonstrated this suggestion.

Recently, evidence for the formation of endothelin by red blood cells from endogenous precursor was given, thus suggesting that red blood cells may modulate the vascular tone both directly, by releasing ATP or endothelin-1, and indirectly, when hemolysis occurs and hemoglobin is released. So far, the pathophysiological significance of these findings has not been well established in hypertension, but it is reasonable to hypothezise clinical implications for the pathogenesis and the progression of the vascular damage during the hypertensive state.

LORCA, a new versatile hemorheological instrument

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The Laser-assisted Optical Rotational Cell Analyzer (LORCA, Mechatronics, Hoorn, The Netherlands) was originally developed for the quantitative measurement of red blood cell (RBC) deformability based on ektacytometry and laser-diffraction analysis of the cell elongation. The results, expressed as Elongation Index (EI), were plotted versus the applied shear stress yielding the Deformation Curve (DC). Further analysis of the S. Shaped DC revealed other parameters like EI_{max}, EI_{min}, τ (shear stress at the inflection point and a parameter in determining the slope at intermediate stress.

In a later stage, the addition of a backscatter light intensity analyzer enabled the measurement of RBC aggregation, also an important hemorheological cellular parameter, with the same instrument (*Clin. Hemorheol.* **14** (1994), 605–618). Besides the measurement of the total extent of aggregation, the technique enabled various kinetic aspects of this process as well as an estimation of the aggregation tendency, i.e., the minimal stress needed to prevent aggregation.

Since (the measurement of) RBC deformability can be influenced by variation of external factors, like osmolality, pH, medium viscosity and, in the case of sickle cells, by pO_2, additional applications are described by scanning the deformability of RBC subjected to a gradient of one of these external factors. Best known is the Osmoscan, yielding additional information regarding the mechanism of eventually impaired RBC deformability. We have shown that a so-called Viscoscan, identifying a threshould medium viscosity beyond which, due to numbing of the cells, no elongation can be measured, gives a fair estimation of the intracellular viscosity. Recently, Baskurt and Meiselman (*Biorheology* **33** (1996), 489–503) have applied a feature of the LORCA's aggregation mode, i.e., the abrupt cessation of shear followed by analysis of light reflection intensity changes, to determine RBC shape recovery time constant.

In conclusion, the LORCA offers the hemorheologist the possibility to analyze in a sensitive and reproducible way, various hemorheological parameters with one and the same instrument. The instrument has the potential for still more relevant applications like the effect of well defined shear on various processes, e.g., shear induced platelet aggregation.

Morphological approach to red cell deformability in a clinical environment

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We think it is interesting to revalue a method of morphological observation with optical microscope (Zipursky) which is easy to perform even on a large scale in clinical cases. This method is based on making the red cells rigid immediately after the withdrawal and then in the 3-dimensional observation with the optical microscope. In this way it is possible to show that the red cells can be basically divided into two main sub-categories (discocytes and bowls) which represent almost the total normal population, and a few particular shapes, which can be considered pathological (acanthocytes, echinocytes, dacrocytes, keratocytes, knizocytes).

In normal conditions bowls prevail on discocytes, while observations made in conditions of hemorheological deterioration prove that the ratio between these two shapes can reverse.

Can we consider, then, some conditions of hyperviscosity as caused by a modification of the ratio between these subpopulations of red cells? Our experience, in several pathological situations with peripheral ischaemia, in basal conditions and after stress, ischaemia or venous stasis, would confirm this hypothesis while in other pathologies in which hyperviscosity does not depend on red cells but on plasma (paraproteinaemias) the ratio bowls/discocytes is unchanged and the number of altered forms significantly increases. A particular case is acute hepatic insufficiency where we have a significant increase of echinocytes. Various experiences demonstrate that these forms are not stable and are inclined to modify dynamically: they can also be reverted after therapy. Out of curiosity can be shown some examples of red cell morphology in different animal species which testify to the deep diversity among the various red cells which can be found in nature.

Comparison of two laser light scattering methods for study of erytrocyte deformability

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The deformation of RBCs plays a key role in blood circulation. One of the main reasons is that RBCs have to deform to pass through capillaries whose diameter can be smaller than the size of RBCs. In another way, clinical observations revealed reduced RBCs deformability in some diseases like diabetes, hypertension, myocardial infarction, vascular diseases, sickle cells.

Many techniques have been proposed to measure RBCs deformability. We can cite, for example, (1) High shear viscometer: the principle of this techique is based on the hypothesis that at high shear rate, RBCs are completely dissociated and orientated in the flow and the only factor that could influcence blood apparent viscosity would be RBC deformability. Thus by observing the variation of blood apparent viscosity at high shear rate, we can approach RBC deformability; (2) RBC filtration [1]: this method is widely used to measure RBC deformability. The time for a given quantity of RBCs to pass through

a membrane with micropores at a given pressure drop can be an index of RBC deformability; (3) Rheoscope [2]: is direct observation method of the shape of RBC under given shear forces; (4) Centrifugation and micropipette aspiration were also used to study RBC deformation.

More recently, two techniques based on laser diffraction were developed: one is the Laser assisted Optical Rotational Cell Analyser (LORCA, Mechatronics, Amsterdam, The Netherlands) [3] and the other is the laser Shear Stress Diffractometer (RHEODYN SSD, Myrenne, Roetgen, Germany) [4]. These two devices seem to be suitable to approach cell deformation under different shear conditions.

The objective of this work was compare the measurements of the deformability of normal and hardened RBCs by using these two instruments.

Experiments were carried out on 46 healthy human subjects. The elongation index (EI) of normal and hardened RBC (obtained by heating blood at 49°C or by incubating RBC in solutions of diamide) was also measured. The results showed that the standard deviations of the experimental data for normal RBCs were relatively small, especially at high shear stresses (more than 3.0 Pa), but higher than those reported before.

Some correlations between the results given by the two instruments were also found. It should be noted that for hardened RBCs, the standard deviations of the measurements were important compared with the mean values in the two instruments.

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Microcirculation and Laser Doppler

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The Doppler Laser is an instrument used for the quantitative and dynamic study of blood circulation in tissues, most commonly in the skin. Only in the last few years has the Doppler Laser been used for clinical problems.

This technique offers a series of advantages: it permits findings that are objective, continuous, immediate and non-invasive. Injections and other manoeuvers that could interfere with the phenomenon being studied are not needed.

The physical principal on which the methodology is based is called the Doppler Effect. The monochromatic light of the laser uniformly illuminates a small part of tissue containing numerous tiny blood vessels in which cellular blood flow is “stochastic”. The “flux” signal proportionally indicates the cellular blood flow of the area under examination. There is, however, no variation in the amount of non-cellular blood flow at the same rate and corpuscular concentration.

The size of the area under examination depends on the optic charateristics of the tissue; a consequence of the depth of penetration of the laser beam. In skin it is estimated to be roughly half of a 1 mm ray sphere when it is placed in front of the probe. It may be considerably larger in other structures like the walls of the stomach and the intestine.

The spontaneous variability of the signal levels is less than 6% *in vitro*. This may change dramatically when live skin is under examination. Cutaneous perfusion in known to change considerably over

time, even in physiological conditions, due to the extreme sensitivity of the skin circulation to both internal and external stimuli. It is therefore necessary to control these conditions in order to avoid the low reproducibility of results. The problem is generally reduced by checking circulatory activation.

We used the Doppler Laser to examine diseases of a vasospastic nature, to measure the segmentary pressure of the limbs, to assess the effects of vasoactive substances immediately after administration and to observe the timing of circulatory changes in particular areas.

The findings that we obtained during our studies have been subject of papers that we have presented at both national and international conferences.

We paid particular attention to the findings obtained using the Doppler Laser on limbs affected by chronic obliterative arteriopathy. Given that a deterioration in the base-line values is clearly evident only in critical cases, we used various types of vaso-kinetic stress with the intention of inducing occurrence. Distinct anomalies became evident in response to thermal stimuli and to passive postural variations of the limbs.

The improvement and the systematic execution of the post ischaemic hyperaemia test on our patients allowed us to identify precocious microcirculatory deterioration.

Current approaches to non-invasive optical oximetry

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Optical techniques are receiving a growing interest in the field of the determination of biological parameters and, particularly, of tissue oxygenation,since they can be extensively applied *in vivo* in an incruent and non-invasive way.

For long-term monitoring of patient's condition under anesthesia or in intensive care units or after a surgical operation, the so-called pulse oximetry is widely used. Two LEDs, one emitting at around 940 nm and the other one at around 660 nm, are mounted in contact with the skin within a plastic clip placed on the toe, finger or ear lobe. The optical absorption of oxygenated hemoglobin is strong at 660 nm and 100 times weaker at 940 nm: a change in the ratio of the light transmission at the two wavelengths is an index of a cumulative blood oxygenation change. The signal is obviously pulsed at the systolic rate.

In order to dotain, not an index but the value of the oxygen saturation of hemoglobin in living tissues, the near infrared diffuse reflectance is at present the most promising optical technique. In this case two selected laser diode wavelengths, around 750 nm and 810 nm, respectively, are weakly but selectively absorbed by hemoglobin, the contribution of other absorbers being negligible. A proper choice of the laser wavelength near 810 nm is mandatory, since there lies the isosbestic point of hemoglobin, i.e., the wavelength of identical extinction coefficient of hemoglobin and oxyhemoglobin; this choice dramatically simplifies the algorithms to be implemented in order to extract the saturation value from rough data. This technique gets information deep in the tissue and not only from the skin. Indeed the light, emerging from the tissue a few centimeters apart from the impinging point on the skin, is collected by the detector after a diffusion path within a some centimeters depth in the underlying tissue. The most common experimental procedures to obtain the value of oxygen saturation from the diffuse reflectance data are the frequency-domain analysis and the multiplexed continuous wave technique.

Preliminary data using non-invasive optical oximetry in humans

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There are different ways to study the tissular oxygenation in clinical and/or experimental conditions. They can be invasive (IT) or non-invasive techniques (NIT). The ITs use a needle to take an arterial blood sample and to evaluate the arterial O_2 pressure (paO_2) with hemogasanalysis or a probe with a needle to evaluate directly pO_2 in the tissues. As NIT the pulse oximeter (PO), related to the heart rate, is commonly used. It controls patient oxygenation during anesthesia, in operating theatres or in intensive care units and it provides only an index of total blood oxygenation change. This methodology is a non polarographic technique. Another NIT to detect tissular oxygenation is the transcutaneous oximetry (TOX). This is a polarographic technique. We began to use TOX six years ago. We use transcutaneous oximeters (TO) with Combi Sensor to detect both pO_2 and pCO_2. For pO_2there is a platinum cathode $(-)$ placed next to an Ag/AgCl anode $(+)$ steeping in a phosphate buffer and potassium chloride solution. For pCO_2 a glass pH cathode $(-)$ and the same Ag/AgCl anode $(+)$ are used. The pO_2 is measured with a galvanometer, the pCO_2 using a potentiometer. In order to obtain not only an index but the value of oxygen saturation of hemoglobin in living tissues we use a new experimental instrument: the optical oximeter (LOX). The LOX uses 2 LEDs, like the pulse oximeter (PO). One LED operates at 670 nm (Hb absorption), the other one operates at 830 nm, i.e., near the isosbestic point for which Hb and HbO_2 show the same extinction. The oxygen saturation of Hb can be expressed as a linear function of the ratio of the absorption coefficients at the wavelengths of the 2 LEDs. Indeed the LOX is based on diffused reflectance measurements and not on light transmission such as the POs. We studied in standard conditions 10 healthy volunteer subjects (6M+4F aged 40 ± 4 years) non smokers. We used TO (Combi Sensor) at the suclavicular standard area and at the right ankle, PO with the probe always at 2nd finger of the right hand and LOX at right wrist and at right ankle.

We obtained a significant relationship between values of TO, PO and LOX ($p < 0.01$).

Our preliminay data suggest that this could be a new interesting methodology to evaluate tissular oxygenation, also exploring living tissues several centimeters deep.