Human clinical hemorheological studies in healthy subjects and in patients with coronary artery disease

PhD dissertation

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<tr>
<td>ACEI</td>
<td>angiotensin-converting enzyme inhibitor</td>
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<td>AFRW</td>
<td>alcohol free red wine extract</td>
</tr>
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<td>AI</td>
<td>LORCA aggregation index</td>
</tr>
<tr>
<td>ARB</td>
<td>angiotensin-receptor blocker</td>
</tr>
<tr>
<td>ASA</td>
<td>acetylsalicylic acid</td>
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<td>BMI</td>
<td>body mass index</td>
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<tr>
<td>CABG</td>
<td>coronary artery bypass grafting</td>
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<td>CAD</td>
<td>coronary artery disease</td>
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<td>CHD</td>
<td>coronary heart disease</td>
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<tr>
<td>CLP</td>
<td>clopidogrel</td>
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<td>CT</td>
<td>computed tomography</td>
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<td>CV</td>
<td>cardiovascular</td>
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<td>CVD</td>
<td>cardiovascular disease</td>
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<td>DM</td>
<td>diabetes mellitus</td>
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<tr>
<td>EI</td>
<td>elongation index</td>
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<tr>
<td>Hct</td>
<td>hematocrit</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
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<tr>
<td>Hgb</td>
<td>hemoglobin</td>
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<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
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<tr>
<td>LORCA</td>
<td>Laser-assisted Optical Rotational Cell Analyzer</td>
</tr>
<tr>
<td>M</td>
<td>Myrenne aggregation index (M mode)</td>
</tr>
<tr>
<td>M1</td>
<td>Myrenne aggregation index (M1 mode)</td>
</tr>
<tr>
<td>MCH</td>
<td>mean corpuscular hemoglobin</td>
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<tr>
<td>MCHC</td>
<td>mean corpuscular hemoglobin concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>mean corpuscular volume</td>
</tr>
<tr>
<td>NSAID</td>
<td>non-steroid anti-inflammatory drug</td>
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<tr>
<td>PCI</td>
<td>percutaneous coronary intervention</td>
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<td>PTP</td>
<td>pre-test probability</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PV</td>
<td>plasma viscosity</td>
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<td>RBC</td>
<td>red blood cell</td>
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<td>SDR</td>
<td>standardized death ratio</td>
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<tr>
<td>S.E.M</td>
<td>standard error of mean</td>
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<tr>
<td>t½</td>
<td>LORCA aggregation half time</td>
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<td>WBV</td>
<td>whole blood viscosity</td>
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<td>WHO</td>
<td>World Health Organization</td>
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Introduction

The burden of cardiovascular diseases

Cardiovascular diseases (CVDs) are the leading cause of death globally. In 2008, around 17.3 million people died of CVDs, representing 30% of total global deaths, of which an estimated 7.3 million were due to coronary heart disease (CHD). In the high-income countries, cardiovascular (CV) mortality is decreasing due to the improving prevention programs and healthcare system, however in the low- and middle-income countries it increases in a very fast rate. Contrary to popular opinion, over 80% of CV deaths take place in the low- and middle-income countries, thus in spite of the decreasing trend in the high-income countries, the global CV mortality is still expected to rise in the future: by 2030 it is projected to reach 23.3 million and to remain the number one cause of death. Most of these events could be prevented, therefore further research of their risk factors, pathogenesis and prevention is essential. In Hungary – considered as an “upper middle income” country – CV mortality is decreasing since 1993 [standardized death ratio (SDR), 1993: 640.48/100,000], but it is still much higher [SDR, 2011: 402.08/100,000] compared to the European Union members before May 2004 [SDR, 2011: 160.11/100,000] (Mathers & Loncar, 2006; World Health Organization, 2008; World Health Organization, 2011; World Health Organization, 2013; World Health Organization, 2014).

The French paradox

Series of prospective epidemiological studies, such as the Framingham Study (Gordon & Kannel, 1984), the British Doctors Study (Doll, et al., 2005), the Nurses Health Study (Fuchs, et al., 1995; Mukamal, et al., 2005), the Physicians’ Health Study (Gaziano, et al., 2000), the British Heart Study (Emberson, et al., 2005) and the Copenhagen Heart Study (Schnohr, et al., 2002) observed a J-shaped relationship between the relative risk of CHD and alcohol intake. According to these studies, low to moderate consumption of
alcoholic beverages is associated with reduced risk of CHD, while on the other hand, binge or heavy drinking increases the risk of CHD. A meta-analysis reported that light or moderate alcohol consumption may also be protective against total and ischemic stroke, while heavy alcohol consumption increased their relative risk (Reynolds, et al., 2003). According to another meta-analysis, involving 34 studies, consumption of alcohol, up to 2 drinks/day in women and 4 drinks/day in men is inversely associated with total and CV mortality, while higher doses of alcohol are associated with increased mortality (Di Castelnuovo, et al., 2006).

Further studies have described that wine is more beneficial than any other forms of alcohol. According to the Copenhagen City Heart Study, low-to-moderate intake of wine is associated with lower mortality from CV and cerebrovascular diseases, while similar intake of spirits increases and beer drinking does not affect mortality (Gronbaek, et al., 1995). A prospective cohort study, carried out in France, reported that moderate consumption of either beer or wine lowered the risk of CV deaths, but the effect of wine was more significant. In case of all-cause mortality only wine was associated with reduced relative risk (Renaud, et al., 1999). A meta-analysis processing 26 studies found a significant inverse association between low to moderate wine consumption and vascular risk. Beer drinking was also associated with reduced risk of vascular events, but failed to show any significant correlation between different amounts of beer intake and vascular risk (Di Castelnuovo, et al., 2002). Several papers suggest that the greater benefit seen in wine drinkers compared to other alcohol consumers can also be attributed to the wine drinkers’ advantageous lifestyle characteristics (Wannametheem & Shaper, 1999).

The beneficial effect is likely to depend on the type of wine. In spite of the much lower cholesterol levels in Alsace, a white wine drinking region in France, higher mortality rates were observed there, compared to the red wine drinking Mediterranean regions (Opie & Lecour, 2007). Studies examining alcohol free red wine extract (AFRW) also support that red wine has additional positive effects beyond alcohol alone. In 1993 Frankel, et al. found that 1000-fold diluted red wine inhibited copper-catalyzed low density lipoprotein (LDL) oxidation, significantly more than alpha-tocopherol, a known antioxidant. In another study 113 ml of AFRW, but not white wine increased total plasma
antioxidant capacity 50 minutes after its ingestion (Serafini, et al., 1998). In a double-blind, cross-over study either 250 ml of red wine or AFRW decreased arterial stiffness in patients with coronary artery disease (CAD) (Karatzi, et al., 2005).

Total mortality is not, but mortality from CVDs is much lower in France than in other Western-European countries [Fig. 1.1.], although the consumption of saturated fats [Fig. 1.2.] and blood cholesterol level – considered as major CV risk factors – are higher in this country. Furthermore, prevalence of other risk factors such as smoking and hypertension are similar in France as in other developed regions of Europe. According to epidemiological studies, this phenomenon, called as “French paradox”, may be caused by the moderate and regular consumption of red wine [Fig. 1.3.] (St Leger, et al., 1979; Renaud & de Lorgeril, 1992; Renaud, et al., 1999; de Lorgeril, et al., 2002; Opie & Lecour, 2007; World Health Organization, 2014).

![Fig. 1.1. Mortality due to circulatory diseases in Europe, 2009.
Source: World Health Organization, HFA-DB. Updated: April 2014.](image)

Literature data exist that these epidemiological findings are confounded by social, cultural, lifestyle and dietary factors. A cross-sectional survey, carried out at the French MONICA Centers, found that healthy diet and behaviors are more often observed in wine drinkers than in beer or spirit consumers (Ruidavets, et al., 2004). Another published confounding factor is called “time lag” hypothesis which states that in the 1960s fat intake was lower in France than nowadays, and it takes about 25-35 years till dietary changes manifest in mortality data (Law & Wald, 1999).
According to several *in vitro* and *in vivo* studies, the favorable effect of red wine may originate from its alcoholic and non-alcoholic components. Moderate intake of alcohol lowers platelet aggregation and fibrinogen level (Renaud & Ruf, 1996), increases high density lipoprotein (HDL) level (McConnell, et al., 1997), tissue-type plasminogen activator level (Ridker, et al., 1994; Aikens, et al., 1998) and production of endothelial nitrogen monoxide (Abou-Agag, et al., 2005), decreases insulin level and improves insulin sensitivity (Davies, et al., 2002).
Non-alcoholic part of red wine contains polyphenols and anthocyanins. Anthocyanins are responsible for the color of wines, while polyphenols are considered as the main source of cardiovascular protection. From this group resveratrol, catechin and quercetin have been extensively studied and their antioxidant properties have been reported (Frankel, et al., 1993; Rice-Evans, et al., 1996; Kerry & Abbey, 1997; Serafini, et al., 1998). The resveratrol content of red wine depends on vintage year and variety (Edelmann, et al., 2001; Casavecchia, et al., 2007), winemaking technology and winery regions (Goldberg, et al., 1995; Kontkanen, et al., 2005; Avar, et al., 2007). It has been revealed that polyphenols increase production of nitrogen monoxide (Leikert, et al., 2002), inhibit platelet aggregation and decrease the production of proinflammatory eicosanoids (Pace-Asciak, et al., 1995; Soleas, et al., 1997). Polyphenols also decrease the oxidation of LDL (Frankel, et al., 1993; Teissedre, et al., 1996), Apo B100 production and increase the expression of LDL receptors (Pal, et al., 2003).

Hemorheological parameters as cardiovascular risk factors

Decades ago prospective epidemiological studies – such as the Framingham Study – identified the conventional risk factors of CAD: age, male gender, arterial hypertension, smoking, diabetes mellitus, dyslipidemia, obesity, stress, low physical activity and positive family history. Reduction of the modifiable risk factors help to considerably prevent CV events, but not all the CV events can be explained by the presence of the classical risk factors thus further investigation of new risk factors is necessary. Further studies have revealed several additional risk factors: abnormal hemorheological parameters, hyperuricemia, metabolic syndrome, hyperhomocysteinemia, infections (e.g. Chlamydia pneumoniae), chronic inflammation, microalbuminuria, chronic renal failure, oxidative stress, air pollution, noise pollution, carotid intima/media thickness and high resting heart rate.

The alterations of hemorheological parameters in CAD have been described by several prospective epidemiological studies. Moreover the Framingham Study (Kannel,
et al., 1987), the Edinburgh Artery Study (Lowe, et al., 1993; Lee, et al., 1998), the MONICA-Augsburg Cohort Study (Koenig, et al., 1998), the Physicians' Health Study (Ma, et al., 1999), the Caerphilly Study and the Speedwell Study (Yarnell, et al., 1991; Sweetnam, et al., 1997) identified elevated hematocrit (Hct), whole blood viscosity (WBV), plasma viscosity (PV) and plasma fibrinogen level as primary CV risk factors. In the Physicians' Health Study elevated plasma fibrinogen level, independently of other CV risk factors, was associated with increased risk of future myocardial infarct (Ma, et al., 1999). In the Edinburgh Artery Study WBV, PV and fibrinogen level correlated linearly with carotid intima/media thickness even on multivariate analysis. After adjusting all common CV risk factors, elevated WBV and fibrinogen level still significantly correlated with carotid intima/media thickness. These results suggest that altered hemorheological parameters are associated not just with CVDs, but also with the early stages of atherosclerosis (Lee, et al., 1998).

Clinical importance of hemorheological parameters in cardiovascular diseases

In spite of the systemic nature of CV risk factors, atherosclerotic lesions do not occur randomly in the vascular system. These lesions tend to develop at specific locations – at the outer wall of vessel bifurcations, the inlet of branches and the inner, distal wall of arterial curvatures – suggesting the importance of hemodynamic and hemorheological factors in their pathogenesis (Chien, et al., 1987; Toth & Kesmarky, 2007). The fact that hemorheological alterations appear prior to the development of vascular lesions suggests that they could play a role in the pathogenesis of the atherosclerotic process. (Vaya, et al., 1996; Toth & Kesmarky, 2007).

The coronary vessel system is a unique part of the human vascular structure due to the periodic change in perfusion pressure and blood flow. Moreover, it has the narrowest capillaries in the human vascular system, thus hemorheological alterations might have a more significant impact on myocardial perfusion compared to other organs. At normal circumstances coronary blood flow is mainly determined by
hemodynamic factors, but in certain conditions, such as a pre-existing coronary stenosis, alteration of hemorheological parameters may early impair myocardial microcirculation (Dintenfass, 1969; Chien, et al., 1987; Baskurt, et al., 1991; Toth & Kesmarky, 2007).

**Hematocrit**

Hct and its determinants, such as RBC count and mean corpuscular volume (MCV), affect most of the other hemorheological parameters. Both low and high Hct can impair tissue oxygen supply by either lowering the oxygen binding capacity or increasing WBV and flow resistance. Elevated Hct has been identified as a CV risk factor by several epidemiological studies. Furthermore, epidemiological data suggest that increased Hct even within the normal range is a risk factor of acute myocardial infarct (Toth & Kesmarky, 2007).

**Plasma and whole blood viscosity**

Due to the axial migration of RBCs, the mechanical influence of blood flow is mediated through the plasma. PV is an important parameter determining wall shear stress (Cabrales & Tsai, 2006) and flow resistance in the microcirculation (Meiselman & Baskurt, 2006). It has also been demonstrated that PV plays a significant role in the regulation of vascular tone (Tsai, et al., 1998; Cabrales & Tsai, 2006).

WBV is a major determinant of flow resistance. In case of high WBV, flow resistance increases, while flow rate decreases proportionally. To maintain adequate flow, elevated blood pressure and cardiac work is required. The increased stress both to the heart and the vessels promote cardiac and vascular remodeling, leading to heart failure and accelerated atherosclerosis. Elevated WBV has also been identified as a CV risk factor by numerous epidemiological studies.

**Red blood cell aggregation**

At constant Hct and temperature, RBC aggregation is the primary determinant of low shear blood viscosity (Cokelet & Meiselman, 2007). Enhanced RBC aggregation alters
both macro- and microrheological properties of blood, and can either decrease or increase flow resistance at different sites of the circulation (Baskurt & Meiselman, 2007). RBC aggregation is determined by several factors: by membrane surface adhesion molecules, type and concentration of macromolecules in the plasma, such as fibrinogen. Alteration of any of the mentioned factors alters RBC aggregation and thus blood flow characteristics (Cokelet & Meiselman, 2007).

**Red blood cell deformability**

Deformability of RBCs is essential to pass through the coronary capillaries being narrower than the cell itself. As mentioned earlier, coronary capillaries are the narrowest in the human body, therefore perfusion insufficiency due to impaired RBC deformability may firstly manifest in the myocardium. RBC deformability impairment may seriously reduce myocardial oxygen supply even in case of normal epicardial vessels by increasing flow resistance in the capillaries (Baskurt & Meiselman, 2007; Toth & Kesmarky, 2007).
Aims

Atherosclerosis of the coronary arteries starts in the early childhood and progresses asymptotically until a significant stenosis develops or a plaque rupture causes acute coronary syndrome. Hemorheological parameters have been identified as CV risk factors and supposed to play a pathophysiological role in the development of atherosclerosis. Our two studies were designed to investigate the alterations of hemorheological factors in two distant points of the atherosclerotic process: in healthy subjects, on one side and in patients with CAD, on the other side.

Hemorheological effects of moderate red wine consumption

Until now only, a limited number of controlled studies have reported the medium term effects of regular red wine intake on hemorheological parameters in healthy volunteers (Jensen, et al., 2006; Kaul, et al., 2010). These experiments gave information about PV, WBV and RBC deformability, but no literature data have been found about RBC aggregation. Our previous in vitro experiments showed that red wine, AFRW and ethanol significantly and dose dependently decrease RBC aggregation (Rabai, et al., 2010; Rabai, et al., 2014). In our current study we aimed to confirm the in vitro findings and examine the effects of moderate red wine consumption on hemorheological parameters, including viscosity, RBC aggregation and deformability. Ours is the first prospective, controlled study which examined a complete hemorheological panel in this field.

Hemorheological parameters in CT-detected coronary artery disease

Previous clinical studies reported significantly elevated Hct, WBV, PV, fibrinogen level and RBC aggregation in CAD (Lowe, et al., 1980; Rainer, et al., 1987; Lee, et al., 2008),
however only a few were able to detect statistically significant differences between the various stages of CAD (Neumann, et al., 1989; Kesmarky, et al., 1998).

Our previous study showed significantly increased Hct, WBV, PV and fibrinogen levels in CAD compared to healthy controls, moreover significant differences were found between CAD subgroups, suggesting a correlation with the severity of CAD (Kesmarky, et al., 1998).

We aimed to conduct a full scale hemorheological study on patients with CAD, including the measurement of RBC aggregation and deformability which was not carried out previously. To the best of our knowledge, all previous studies used invasive coronary X-ray angiography to evaluate for CAD. In our current study, a new and well established method, coronary CT angiography was used for the evaluation of the coronary vessel system. This method is able to measure coronary stenosis precisely and can also determine the total extent of coronary calcification.
Hemorheological effects of moderate red wine consumption

Methods

Subjects

The study was approved by the Regional Ethics Committee of the University of Pecs and written informed consent was signed by all subjects. Forty healthy, non-smoking male volunteers between the ages of 18-40 were enrolled. None of them had taken any drugs for one week prior entering the study and during the whole duration of it. In order to eliminate any possible effect of the previously consumed alcoholic beverages, no alcohol consumption was allowed in the first 7 days of the study. On the morning of the 8th day the subjects were assigned into 2 groups:

- In the control group, volunteers drank mostly water for 3 weeks, coffee and soft drinks were permitted, no alcohol consumption was allowed.
- In the red wine group, 2 dl of red wine (2007 Merlot, Polgar Winery, Villany, Hungary; resveratrol content \( \approx 4 \text{ mg/l} \), ethanol content \( \approx 13.5 \text{ V/V\%} \)) was consumed each day during dinner for 3 weeks, no other forms of alcoholic beverages were allowed [Fig. 2.1].

[Fig. 2.1. Study protocol.]

<table>
<thead>
<tr>
<th>40 healthy young subjects</th>
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<tr>
<td>1st - 7th days: no alcohol consumption</td>
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<tr>
<td>8th day: baseline blood samples</td>
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<tr>
<td>Control group</td>
</tr>
<tr>
<td>- No alcohol</td>
</tr>
<tr>
<td>- Mostly tap water</td>
</tr>
<tr>
<td>- Soft drinks and coffee allowed</td>
</tr>
<tr>
<td>Red wine group</td>
</tr>
<tr>
<td>- 2 dl of red wine for dinner</td>
</tr>
<tr>
<td>- No other alcohol</td>
</tr>
<tr>
<td>- Additional fluid intake similarly to control group</td>
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<tr>
<td>29th day: 3-week blood samples</td>
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Fig. 2.1. Study protocol.
Blood sampling

After fasting for 12 hours, in the mornings of the 8th and 29th days, 17 ml of antecubital venous blood samples were obtained into Li-heparin coated BD Vacutainer® tubes with a 21-gauge butterfly needle set with a minimal tourniquet, according to the latest guideline for hemorheological laboratory techniques (Baskurt, et al., 2009). Blood samples were stored at ambient temperature until hemorheological measurements.

Hemorheological measurements

Hemorheological measurements were performed within 1 hour from blood sampling at the Hemorheological Laboratory of the 1st Department of Medicine, University of Pecs Medical School.

Hematocrit

The anticoagulated blood samples were placed into a native capillary then centrifuged with Haemofuge microhematocrit centrifuge (Heraeus; Germany) at 12,000 RPM for 3 minutes at room temperature (22±1°C). Hct was determined by the fraction of volume occupied by RBCs. For further measurements Hct was adjusted to 40% with autologous plasma.

Plasma and whole blood viscosity

PV and WBV were measured by Hevimet 40 capillary viscometer (Hemorex; Budapest, Hungary) [Fig. 2.2.]. Plasma was obtained by centrifugation of blood samples at 2500 g for 10 minutes at 22°C (Labofuge 400 R, Heraeus; Germany).

The viscometer is composed of a vertical glass tube and 40 diodes placed next to it. 620 μl of fluid is injected into the system and released to flow out, while the height of the fluid column is registered in the function of time. The height of each diode
from the baseline of the fluid column is known, thus the flow maintaining hydrostatic pressure and shear stress at each point can be calculated. For the same points, flow velocity and shear rate is calculated from the height-time function. From the shear stress and shear rate data points, viscosity values are determined. Viscosity values interpolated at 90 s$^{-1}$ shear rate were used in our study. Measurements were performed at 37 °C temperature.

**Hematocrit per whole blood viscosity ratio**

From Hct and WBV data, *Hct/WBV ratio* was calculated to determine rheological oxygen carrying capacity of blood. Oxygen binding capacity linearly, viscosity and flow resistance exponentially increases with Hct. In healthy subjects Hct/WBV ratio reliably reflects rheological oxygen carrying capacity of blood (Bogar, et al., 2005). Values of Hct/WBV in the function of Hct results in an inverted U-shaped curve [Fig. 2.3.] (Kenyeres, 2010). Both in anemia and polycythemia oxygen supply is impaired and lower Hct/WBV ratios are calculated. In case of anemia, low oxygen transport originates from low oxygen binding capacity, while in polycythemia the decreased flow velocity is responsible for the clinical symptoms. A study from our research group demonstrated that unfavorable Hct/WBV ratio in patients with CHD is associated with increased risk of cardiac morbidity and mortality (Kenyeres, et al., 2008).

**Red blood cell aggregation**

RBC aggregation was measured by *Myренне* (MA-1 Aggregometer, Myrenne GmbH; Roetgen, Germany) and *LORCA* (Laser-assisted Optical Rotational Cell Analyzer; R&R Mechatronics; Hoorn, The Netherlands) aggregometers. Blood samples were taken from the same tube.
**Myrenne aggregometer** [Fig. 2.4.] employs the light transmission method of *Schmid-Schönbein* through a transparent cone-plate shearing instrument (Klose, et al., 1972). 30 µl of blood is injected between the glass cone and plate. The sample is sheared at 600 s\(^{-1}\) to disperse all pre-existing RBC aggregates, then shear rate falls to zero (in M mode) or to 3 s\(^{-1}\) (in M1 mode). The instrument measures the infrared light intensity passing through the blood sample: as RBCs aggregate the light intensity gradually increases. The extent of aggregation was characterized by the aggregation indices (M, M1) calculated from the surface area under the light intensity curve in a 10 s period of time (Vaya, et al., 2003). Measurements were performed at room temperature (22±1 °C).

**LORCA aggregometer** [Fig. 2.5.] detects the laser back-scattering generated by the RBCs. To increase back-scattering, blood samples were oxygenated by incubation with 10-times higher volume of air on a rollerbank for 15 minutes. 1 ml of oxygenated blood was injected into the gap between the static inner glass cylinder and the rotating outer glass cylinder which creates a simple shear flow. RBCs are first disaggregated at 500 s\(^{-1}\) shear rate, then shear rate falls to zero. During RBC aggregation, the intensity of back-scattering laser light is drawn in the function of time (syllectogram) [Fig. 2.6.]. Aggregation behavior of blood was characterized by:
- the aggregation index (AI), calculated from the first 10 seconds of the syllectogram after the shape recovery period: \( AI = A/(A+B) \) [Fig. 2.6.]
- and the time that elapses until peak intensity is reduced by half the amplitude (t½) (Hardeman, et al., 2001; Dobbe, 2003).

During the measurements temperature was kept at 37 °C. Analyses were performed on LORCA Aggregation Program v2.1.

**Red blood cell deformability**

RBC deformability was measured by LORCA [Fig. 2.5.], using the laser diffraction ellipsometry technique. 25 μl of blood was suspended in 5 ml of high viscosity (32.6 mPas) polyvinylpyrrolidone (Sigma-Aldrich, 360 kDa) solution dissolved in phosphate buffered saline (290 mOsm/kg, pH = 7.4). 1 ml of this suspension was injected into the gap between the two cylinders. RBCs are deformed by various shear stresses, generated...
by the rotation of the outer cylinder, meanwhile a laser diode is projecting through the sample. The elongated RBCs create a laser diffraction pattern captured by a video camera and analyzed by *LORCA Elongation Program v2.1*.

RBC deformability was characterized by the elongation index (EI), calculated from the two radiiuses of the ellipsoid diffraction pattern as \((A-B)/(A+B)\), at shear stresses from 30 Pa to 0.3 Pa. From these data the deformability curve was drawn [Fig. 2.7.] (Hardeman, et al., 1994; Dobbe, 2003).

**Statistical analysis**

Continuous variables are presented as mean values ± S.E.M. Differences were analyzed using paired (dependent samples between baseline and 3\(^{rd}\) week results) and un-paired (independent samples between the control and the red wine group) Student’s t-test. A two-tailed p value less than 0.05 was considered statistically significant.

**Results**

**Subjects**

There was no significant difference between the *control* and the *red wine group* in age, body mass index (BMI) and average physical activity [Table 2.1.]. One volunteer from the wine drinking group was withdrawn due to complaints of heartburn.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Red wine group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>27.5 ± 1.2</td>
<td>26.8 ± 1.0</td>
</tr>
<tr>
<td><strong>BMI (kg/m(^2))</strong></td>
<td>24.9 ± 0.87</td>
<td>23.9 ± 0.64</td>
</tr>
<tr>
<td><strong>Physical activity score</strong></td>
<td>3.0 ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
</tbody>
</table>

Hematocrit

Red wine consumption had no effect on Hct and no difference was found between the two groups neither at baseline, nor after 3 weeks [Table 2.2.].

### Table 2.2.
**Hemorheological parameters.**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Red wine</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>47.8 ± 0.6</td>
<td>46.6 ± 0.5</td>
</tr>
<tr>
<td>PV</td>
<td>1.27 ± 0.02</td>
<td>1.25 ± 0.03</td>
</tr>
<tr>
<td>WBV (mPa·s)</td>
<td>3.9 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>Hct/WBV (mPa·s⁻¹)</td>
<td>10.4 ± 0.3</td>
<td>10.7 ± 0.2</td>
</tr>
<tr>
<td>M</td>
<td>8.6 ± 0.7</td>
<td>9.0 ± 0.4</td>
</tr>
<tr>
<td>M1</td>
<td>16.0 ± 0.7</td>
<td>15.2 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>AI</td>
<td>57.2 ± 2.2</td>
<td>55.4 ± 1.7</td>
</tr>
<tr>
<td>t½ (s)</td>
<td>2.9 ± 0.3</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>EI (at 30 Pa)</td>
<td>0.631 ± 0.002</td>
<td>0.630 ± 0.003</td>
</tr>
</tbody>
</table>

<sup>a</sup>: significant (p<0.05) difference compared to the control group at baseline<br>
<sup>b</sup>: significant (p<0.05) difference compared to the red wine group at baseline<br>
<sup>c</sup>: significant (p<0.05) difference compared to the control group after 3 weeks

Viscosity

PV did not change during the 3 weeks in either group compared to baseline, and there was no significant difference between the two groups nor at baseline, neither after 3 weeks [Table 2.2.].

Adjusted WBV remained constant in the control group, while in the red wine group it decreased, and after 3 weeks WBV in the red wine group became significantly lower compared to the control group (p<0.05) [Table 2.2.].
Hematocrit per whole blood viscosity ratio

The Hct/WBV ratio remained steady during the 3 weeks in the control group, while in the red wine group the parameter tended to increase compared to baseline. After 3 weeks, significantly higher (p<0.05) ratio was calculated in the red wine group compared to the control group [Table 2.2., Fig. 2.8.].

![Hct/WBV ratio (40%)](image)

**Fig. 2.8. Change in hematocrit/adjusted whole blood viscosity ratio.**
Arrow represents significant (p<0.05) difference.

Red blood cell aggregation

Both Myrenne (M and M1) and LORCA aggregation indices significantly decreased (p<0.05) in the red wine group [Table 2.2., Fig. 2.9., 2.10.]. LORCA t½ also indicates significantly (p<0.05) decreased RBC aggregation in the red wine group [Table 2.2.]. Furthermore, after 3 weeks, Myrenne M1 parameter was also significantly lower (p<0.05) in the red wine group compared to the control group. M1 significantly decreased (p<0.05) in the control group after 3 weeks, but none of the other RBC aggregation parameters (M, AI, t½) showed a significant change [Table 2.2., Fig. 2.9., 2.10.].
**Red blood cell deformability**

At the highest shear stress (30 Pa), the EI significantly increased (p<0.05) in the red wine group after 3 weeks compared to baseline, while it did not change in the control group [Table 2.2.].
Discussion

Only limited data have been presented on the hemorheological consequences of medium and long-term red wine consumption. Several studies have demonstrated an inverse association between red wine or polyphenol intake and cardiovascular events, but not all sources of this cardioprotective effect are known. Most authors have emphasized the effect of elevated HDL level, decreased platelet aggregation and fibrinogen level, thus these were mostly examined. In a randomized crossover study, five-week red wine consumption significantly decreased collagen-induced platelet aggregation and increased HDL level (Pikaar, et al., 1987), moreover in another study collagen-induced platelet aggregation and fibrinogen level were significantly reduced after 4-week red wine intake (Pellegrini, et al., 1996).

Only a few controlled results are known about other important hemorheological parameters – such as PV, WBV, RBC aggregation and RBC deformability – in healthy human subjects consuming red wine. A randomized controlled study reported that moderate red wine consumption (6 or 12 ounces/day; = 1.77 or 3.55 dl/day), up to 2 weeks, significantly increased HDL level, but it did not change Hct, WBV, RBC deformability and fibrinogen level (Kaul, et al., 2010). Another controlled experiment observed significantly reduced PV and fibrinogen level after 3-week moderate red wine intake (Jensen, et al., 2006). Our prospective, controlled study presents new data about the in vivo hemorheological effects of moderate red wine consumption.

Hematocrit

Red wine consumption had no significant effect on Hct, confirming the results of Kaul, et al. (2010). In order to completely eliminate the influence of Hct on the dependent hemorheological parameters (WBV and RBC aggregation) Hct was standardized to 40%.

Plasma and whole blood viscosity

PV did not change significantly after 3-week red wine consumption. Although we did not measure plasma fibrinogen level (the main determinant of PV), Kaul, et al. (2010)
reported no significant change after 2 weeks of red wine intake. On the other hand, lower plasma viscosity and fibrinogen level were measured after 3 weeks in a different study (Jensen, et al., 2006).

The observed reduction of WBV in the red wine group may result from the reduction of RBC aggregation and the increased RBC deformability. Red wine consumption increased WBV of Hct-standardized samples after 3 hours, but no difference from baseline was observed 13 hours after ingestion (Fehr, et al., 2008). This suggests that the observed reduction of WBV in our study was not due to the short-term effect of the red wine, consumed in the previous evening. Kaul, et al. (2010) found no changes after 2 weeks of red wine consumption, but Jensen, et al. (2006) reported decreased WBV after 3 weeks. It is assumed that more time is required until the effect can be detected.

**Hematocrit per whole blood viscosity ratio**

The significantly higher Hct/WBV ratio in the red wine group (due to unaffected Hct and lowered WBV) means greater oxygen carrying capacity of the blood.

**Red blood cell aggregation**

The reduction of RBC aggregation was observed both by Myrenne and LORCA in the red wine group. These findings confirm our previous in vitro results, where red wine, AFRW and ethanol inhibited RBC aggregation (Rabai, et al., 2010; Rabai, et al., 2014). The decrease of RBC aggregation may be a consequence of the modifications of plasma proteins. It is known that polyphenols, such as resveratrol, are bound to plasma proteins due to their poor water solubility. The phenol-protein interactions may change the properties of plasma proteins and RBC surface molecules, therefore reducing the capability to form cross links between cellular components and decreasing RBC aggregation. It has been reported that resveratrol binding to albumin or hemoglobin changes their secondary structures (Lu, et al., 2007). The absorption of resveratrol in humans is about 70%, but due to the extensive metabolism, resveratrol is mostly present in sulphate and glucuronide conjugated forms and only minimal amounts of
unchanged resveratrol can be detected in the plasma (Walle, et al., 2004). The reported fibrinogen lowering effect of red wine (Jensen, et al., 2006) may also be a reason of the observed decrease in RBC aggregation.

**Red blood cell deformability**

RBC deformability was enhanced at the highest shear stress in the red wine group. El at 30 Pa approximates the maximal deformability of RBCs. In our earlier in vitro study, no significant changes were observed after direct addition of red wine or AFRW to the blood samples (Rabai, et al., 2010). It was assumed that under no significant oxidative stress, RBC of healthy humans has optimal deformability; therefore no further improvement could be expected. On the other hand, RBC deformability even of healthy volunteers could be improved with moderate red wine consumption. Contrarily, another study reported no significant changes in RBC deformability after 2 weeks of red wine consumption (Kaul, et al., 2010). It is possible again, that more time is required till the changes become significant.

**Conclusions**

This in vivo study confirmed our previous in vitro findings about the beneficial hemorheological effects of ethanol, AFRW and red wine on RBC aggregation. Decreased WBV, RBC aggregation, higher calculated oxygen carrying capacity and increased RBC deformability may positively affect microcirculation. These findings may take part in the cardiovascular protection of moderate red wine consumption.
Hemorheological parameters in CT-detected coronary artery disease

Methods

Patients

The study was approved by the Regional Ethics Committee of the University of Pecs. 130 patients, admitted to coronary CT angiography at the Department of Radiology, University of Pecs Medical School, participated in the study. Informed consent was signed by all subjects prior to enrollment. Patients were questioned regarding previous medical history and existing medications. Patients with autoimmune disorders or malignancies were not enrolled.

Blood sampling

Blood samplings were performed according to the latest guideline for hemorheological laboratory techniques (Baskurt, et al., 2009). Samples were obtained via an antecubital vein just before the coronary CT examination, using a 21-gauge butterfly needle set with minimal tourniquet into the following BD Vacutainer® tubes:

- 2x6 ml Li-heparin anticoagulated (for hemorheological measurements)
- 3 ml K₂EDTA anticoagulated (for qualitative and quantitative blood cell analyses)
- 2.7 ml Na-citrate anticoagulated (for fibrinogen level)
- 5 ml SST native (for lipid profile)

Blood samples were stored at ambient temperature until hemorheological measurements.
Coronary computed tomography angiography

Coronary CT angiography examinations were performed at the Department of Radiology, University of Pecs Medical School with a first generation 64-slice, dual source Siemens Somatom Definition CT device. The instrument has two X-ray tubes and dual detector system in perpendicular position: during coronary CT measurements the two systems work simultaneously, providing better temporal resolution, which reduces the presence of motion artefacts (Dérczy & Battyány, 2008).

Prior to the examinations no food or caffeine containing fluid consumption was allowed for 4 hours. Sublingual nitrate was given to all patients and depending on the resting heart rate, intravenous beta-blocker was administered. First a native scan was carried out to estimate total coronary calcification. After that a contrast enhanced scan was performed to evaluate coronary system. Coronary calcification was characterized by Agatson-score; lesions were defined as area stenosis. Data processing and image evaluation was done with Siemens syngo software on Siemens Multimodality workstation (Dérczy & Battyány, 2008).

Hemorheological measurements

Hemorheological measurements were performed within 2 hours from blood sampling at the Hemorheological Laboratory of the 1st Department of Medicine, University of Pecs Medical School. The instruments and measurement protocols used in this study are identical to the ones in the previous work, therefore only the differences will be detailed here:

- Hct, PV and WBV were measured with the same settings.
- RBC aggregation was determined only by Myrenne aggregometer. Both native samples and suspensions of RBCs, adjusted to 40% Hct with autologous plasma were measured. Instrument settings were identical.
- For RBC deformability measurements, blood was suspended in 29.43 mPas viscosity polyvinylpyrrolidone solution. Other conditions remained unchanged.
Central laboratory measurements

Central laboratory measurements were carried out at the Department of Laboratory Medicine, University of Pecs Medical School. The following parameters were measured: Hct, Hgb concentration, RBC count, MCV, MCH, MCHC, LDL, HDL and fibrinogen level.

Classification of patients

Patients were classified into four groups based on their coronary vessel state, according to Table 3.1.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative</strong> (Neg)</td>
<td>no coronary stenosis or atherosclerotic lesion and zero calcium-score</td>
</tr>
<tr>
<td><strong>Non-significant</strong> (NS)</td>
<td>below 40% area stenosis on one or more coronary vessels and no PCI or CABG</td>
</tr>
<tr>
<td><strong>Single-vessel</strong> (SV)</td>
<td>1. over 40% area stenosis on one coronary vessel or 2. history of PCI or CABG on one coronary vessel</td>
</tr>
<tr>
<td><strong>Multi-vessel</strong> (MV)</td>
<td>1. over 40% area stenosis on multiple coronary vessels or 2. over 40% area stenosis on one coronary vessel and history of PCI or CABG on one coronary vessel or 3. history of PCI or CABG on multiple coronary vessels</td>
</tr>
</tbody>
</table>

Statistical analysis

Continuous variables are presented as mean ± S.E.M. Discrete variables are described through relative or absolute frequencies. Continuous variables were analyzed with one-way ANOVA; in case of dichotomous variables χ² test was used. A two-tailed p value less than 0.05 was considered statistically significant.
Results

Nine patients were excluded from the study due to classification-limiting CT image quality.

Subject characteristics

There is an increasing trend in average age, BMI and proportion of males in Negative<Non-significant<Single-vessel<Multi-vessel rank order. The average age in the Multi-vessel group is significantly higher compared to the Negative group. The proportion of males is greatly higher in the CAD (Non-significant, Single-vessel and Multi-vessel) groups compared to the Negative group, and continues to increase in a smaller extent in the CAD subgroups [Table 2.2].

The prevalence of hypertension, diabetes mellitus (DM) and smoking has a similar rank order: Negative<Non-significant<Single-vessel<Multi-vessel. In the Single-vessel and Multi-vessel groups all of them are significantly higher compared to the Negative group. PCI and CABG are only present in the Single-vessel and Multi-vessel groups, being highest in the Multi-vessel group. Ca-score also has the previously described trend [Table 2.2].

| Table 2.2. Demographic data, Ca-score, risk factors and revascularization procedures. |
|-------------------------------------------------|---|---|---|---|
| N   | Neg | NS | SV | MV |
| Age (years) | 56.8 ± 2.0 | 59.2 ± 11.4 | 58.8 ± 1.5 | 62.1 ± 1.5a |
| Males | 22 % | 70 %a | 75 %a | 90 %a |
| BMI (kg/m²) | 27.6 ± 0.9 | 29.3 ± 0.9 | 29.5 ± 0.8 | 29.7± 1.0 |
| Ca-score | 0 | 145.7a | 264.9a | 425.8a,b |
| Hypertension | 66 % | 70 %a | 87 %a | 90 %a |
| DM | 6 % | 22 % | 35 %a | 35 %a |
| Smoking | 19 % | 33 % | 43 %a | 45 %a |
| PCI | 0 % | 0 % | 19 %a,b | 33 %a,b |
| CABG | 0 % | 0 % | 6 % | 30 %a,b,c |

a: significant (p<0.05) difference compared to the Negative group
b: significant (p<0.05) difference compared to the Non-significant group
c: significant (p<0.05) difference compared to the Single-vessel group
The use of antiplatelet, statin, angiotensin-converting enzyme inhibitor (ACEI), angiotensin-receptor blocker (ARB) and beta-blocker medication is already considerable in the Negative group and follows an increasing trend. The prevalence of acetylsalicylic acid (ASA), clopidogrel (CLP), statin and beta-blocker medication in the Multi-vessel group is significantly higher compared to the Negative group. Statin use is significantly higher in all CAD subgroups compared to the Negative group. Dual anti-platelet therapy is only used in the Single-vessel and the Multi-vessel groups.

Table 2.3. Medications.

<table>
<thead>
<tr>
<th>Antiplatelet agents:</th>
<th>Neg</th>
<th>NS</th>
<th>SV</th>
<th>MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono antiplatelet therapy</td>
<td>41  %</td>
<td>62  %</td>
<td>55  %</td>
<td>57  %</td>
</tr>
<tr>
<td>Dual antiplatelet therapy</td>
<td>0  %</td>
<td>0  %</td>
<td>6  %</td>
<td>21 %&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ASA</td>
<td>38  %</td>
<td>46  %</td>
<td>58  %</td>
<td>68 %&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CLP</td>
<td>3  %</td>
<td>15  %</td>
<td>10  %</td>
<td>32 %&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Statin</td>
<td>31  %</td>
<td>62 %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58 %&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64 %&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACEI/ARB</td>
<td>59  %</td>
<td>62  %</td>
<td>68  %</td>
<td>79  %</td>
</tr>
<tr>
<td>Beta-blocker</td>
<td>47  %</td>
<td>63  %</td>
<td>61  %</td>
<td>75 %&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a: significant (p<0.05) difference compared to the Negative group
b: significant (p<0.05) difference compared to the Non-significant group
c: significant (p<0.05) difference compared to the Single-vessel group

Hemorheological parameters

Hematocrit

Hct increases in a Negative<Non-significant<Single-vessel<Multi-vessel manner. In the Non-significant, Single-vessel and Multi-vessel groups it is significantly higher compared to the Negative group [Table 2.4., Fig. 3.1.].

Plasma and whole blood viscosity

No significant difference was found in PV [Table 2.4.]. WBV shows the following rank order: Negative<Non-significant<Single-vessel<Multi-vessel. WBV in the Multi-vessel group is significantly higher compared to the Negative group [Table 2.4., Fig. 3.2].
Table 2.4. Hemorheological parameters.

<table>
<thead>
<tr>
<th></th>
<th>Neg</th>
<th>NS</th>
<th>SV</th>
<th>MV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>42.7 ± 0.4</td>
<td><strong>44.1 ± 0.7</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.3 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.9 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PV (mPa·s)</td>
<td>1.31 ± 0.2</td>
<td>1.28 ± 0.2</td>
<td>1.29 ± 0.2</td>
<td>1.27 ± 0.2</td>
</tr>
<tr>
<td>WBV (mPa·s)</td>
<td>4.3 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td><strong>4.5 ± 0.1</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M</td>
<td>5.5 ± 0.3</td>
<td>5.7 ± 0.3</td>
<td>6.1 ± 0.3</td>
<td><strong>6.4 ± 0.3</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M (40 %)</td>
<td>5.9 ± 0.3</td>
<td>6.1 ± 0.4</td>
<td>6.6 ± 0.3</td>
<td><strong>7.0 ± 0.4</strong>&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>M1</td>
<td>12.3 ± 0.3</td>
<td>12.6 ± 0.5</td>
<td>12.8 ± 0.4</td>
<td>13.0 ± 0.4</td>
</tr>
<tr>
<td>M1 (40 %)</td>
<td>12.8 ± 0.4</td>
<td>12.9 ± 0.6</td>
<td>13.5 ± 0.4</td>
<td>14.0 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EI (at 30 Pa)</td>
<td>0.624 ± 0.002</td>
<td><strong>0.620 ± 0.001</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.622 ± 0.001</td>
<td>0.621 ± 0.002</td>
</tr>
<tr>
<td>EI (at 16.8 Pa)</td>
<td>0.594 ± 0.002</td>
<td><strong>0.590 ± 0.001</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.592 ± 0.002</td>
<td>0.591 ± 0.003</td>
</tr>
</tbody>
</table>

a: significant (p<0.05) difference compared to the Negative group
b: significant (p<0.05) difference compared to the Non-significant group
c: significant (p<0.05) difference compared to the Single-vessel group

**Fig. 3.1. Hematocrit.** Arrows represent significant (p<0.05) differences.

**Fig. 3.2. Whole blood viscosity at 90 s<sup>-1</sup> shear rate.** Arrow represents significant (p<0.05) difference.
Red blood cell aggregation

In native samples, both M and M1 parameters increase in Negative<Non-significant<Single-vessel<Multi-vessel manner. The M parameter is significantly higher in the Multi-vessel group compared to the Negative group [Table 2.4., Fig. 3.3.].

In case of adjusted samples, the M and M1 parameters show similar Negative<Non-significant<Single-vessel<Multi-vessel trend. Both indices are significantly higher in the Multi-vessel group compared to the Negative group, moreover the M parameter is significantly higher in the Multi-vessel group compared to the Non-significant group [Table 2.4., Fig. 3.4.].

Red blood cell deformability

RBC deformability shows a decreasing trend at all shear stresses. EI at 30 and 16.87 Pa was significantly lower in the Non-significant group compared to the Negative group [Table 2.4.].

Fig. 3.3. Red blood cell aggregation in native and adjusted (Hct=40%) samples measured by Myrenne. Arrows represent significant (p<0.05) differences.
Central laboratory parameters

RBC count, Hbg concentration, MCV, MCH, and MCHC have the same rank order: Negative<Non-significant<Single-vessel<Multi-vessel. In the Multi-vessel group all parameters are significantly higher compared to the Negative group. LDL levels are similar in all groups. HDL has a decreasing trend and it is significantly lower in the Multi-vessel group compared to all the other ones. Fibrinogen level has an increasing tendency in the CAD subgroups, although no significant difference was found [Table 2.5.].

Table 2.5.
Central laboratory parameters.

<table>
<thead>
<tr>
<th></th>
<th>Neg</th>
<th>NS</th>
<th>SV</th>
<th>MV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBC count (G/l)</strong></td>
<td>4.5 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>4.7 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Hbg concentration (g/l)</strong></td>
<td>134.7 ± 1.5</td>
<td><strong>140.6 ± 2.2</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>140.5 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.3 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>MCV (fl)</strong></td>
<td>90.5 ± 0.7</td>
<td>91.0 ± 1.0</td>
<td>91.7 ± 1.2</td>
<td>92.2 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>MCH (pg)</strong></td>
<td>30.0 ± 0.3</td>
<td>30.5 ± 0.4</td>
<td>30.7 ± 0.5</td>
<td>31.0 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><strong>MCHC (g/l)</strong></td>
<td>331.0 ± 1.4</td>
<td>334.7 ± 2.2</td>
<td>334.8 ± 2.4</td>
<td>336.6 ± 2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>LDL (mmol/l)</strong></td>
<td>3.1 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>3.1 ± 0.3</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td><strong>HDL (mmol/l)</strong></td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td><strong>1.2 ± 0.1</strong>&lt;sup&gt;a,b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>fibrinogen (g/l)</strong></td>
<td>3.06 ± 0.10</td>
<td>2.97 ± 0.20</td>
<td>3.04 ± 0.11</td>
<td>3.28 ± 0.16</td>
</tr>
</tbody>
</table>

<sup>a</sup>: significant (p<0.05) difference compared to the Negative group
<sup>b</sup>: significant (p<0.05) difference compared to the Non-significant group
<sup>c</sup>: significant (p<0.05) difference compared to the Single-vessel group

Fig. 3.4. Red blood cell aggregation in native and adjusted (Hct=40%) samples measured by Myrenne. Arrow represents significant (p<0.05) difference.
Discussion

Subject characteristics

Age, male gender, obesity, hypertension, DM and smoking are major CV risk factors, thus the Negative<Non-significant<Single-vessel<Multi-vessel trend was expected. The high prevalence of hypertension in the Negative group can be explained by the relatively high average age of this cluster. The same increasing trend was observed in the prevalence of PCI and CABG procedures. These procedures are indicated in hemodynamically significant coronary stenosis, therefore they are only present in the most severe CAD subgroups.

Generally, the amount of applied medication increases with the deteriorating coronary status. The use of anti-platelet, statin, ACEI/ARB and beta-blocker is already notable in the Negative group and increases in the CAD subgroups. According to the latest guideline for the management of CAD, prescription of these drugs is indicated in all cases, if contraindications are not present (The Task Force on the management of stable coronary artery disease of the European Society of Cardiology, 2013). Unfortunately, in the CAD subgroups not all patients receive proper medication. These drugs are also present in the Negative group. It is likely that these patients receive these drugs due to existing hypertension (ACEI/ARB, beta-blocker) or dyslipidemia (statin). Dual antiplatelet therapy is only present in the Single-vessel and Multi-vessel groups, where PCIs were performed and dual therapy is routinely used.

Hematocrit

Hct was significantly higher in the CAD subgroups compared to the patients with no vessel disease which result is similar to the findings of our earlier study (Kesmarky, et al., 1998). However, we were not able to detect significant differences between CAD subgroups, although the increasing tendency is well visible. Elevated Hct has also been reported by Lowe, et al. (1980) and Rainer, et al. (1987). On the other hand Pfafferott, et al. (1999) did not find significant difference between healthy controls, patients with
stable/unstable angina and acute myocardial infarct, but the epidemiological studies strongly support our findings.

It has been demonstrated, that vascular mechanisms can reduce flow resistance increment, caused by increased Hct in isolated dog hind limb (Whittaker & Winton, 1933). Baskurt, et al. (1991) reported, that 57% increase of Hct elevated coronary flow resistance only by 8% in healthy dogs, demonstrating the substantial coronary autoregulatory reserve. However, after exhausting the coronary reserve by inducing critical stenosis, coronary flow resistance increased by 22%. This result suggests that in case of an existing stenosis – e.g. in severe CAD, where hemorheological alteration are greater – altered hemorheological parameters have more impact on the coronary circulation.

**Plasma and whole blood viscosity**

Our study found similar PV levels in all groups, supporting the findings of Lowe, et al. (1980). On the other hand, our earlier study (Kesmarky, et al., 1998), Rainer, et al. (1987), Pfafferott, et al. (1999) and Lee, et al. (2008) found significantly elevated PV in CAD.

No significant difference was found in fibrinogen level, although an increasing trend is evident in CAD subgroups. Lowe, et al. (1980) reported similar plasma fibrinogen levels in CAD compared to healthy controls, while Kesmarky, et al. (1998) and Rainer, et al. (1987) observed a significant increase. The dissimilar findings in PV and fibrinogen levels may originate from the nowadays widely used statin medication. Ten years ago in the same region Marton, et al. (2003) reported only 13% statin medication in patients admitted to hospital due to acute coronary syndrome. This is two times lower compared to our Negative group and four times lower compared to the CAD subgroups.

WBV was significantly elevated in the most severe vessel state, confirming our previous result (Kesmarky, et al., 1998). This result is supported by the findings of Lowe, et al. (1980), Rainer, et al. (1987) and Lee, et al. (2008). Kesmarky, et al. (1998) and Lee, et al. (2008) reported statistically significant differences between CAD subgroups, while Vosseler, et al. (2012) found no significant difference between CAD and healthy controls.
Increased WBV has also been observed in hypertension and diabetes mellitus, therefore the elevated WBV in the Multi-vessel group may be a consequence of the increased prevalence of these comorbidities.

**Red blood cell aggregation**

RBC aggregation was significantly increased in CAD and significant difference was found between the subgroups. Rainer, et al. (1987), Pfafferott, et al. (1999) and Lee, et al. (2008) confirm our results.

RBC aggregation alters both macro- and microrheological properties of blood and can either decrease or increase flow resistance. These effects depend on several factors: vessel diameter, geometry, orientation or flow rate. On one hand, enhanced RBC aggregation reduces flow resistance by increasing axial migration of RBCs, promoting Fahraeus effect and plasma skimming (Baskurt & Meiselman, 2007).

On the other hand, elevated RBC aggregation increases low shear viscosity which may affect flow resistance in large blood vessels when shear forces are sufficiently low. RBC aggregation also has impact at the microcirculatory level: aggregates must be dispersed in order to enter the microcirculation. In case of elevated RBC aggregation, higher force is required which increases microvascular flow resistance (Baskurt & Meiselman, 2007). Studies have also demonstrated *in vivo*, that RBC clumps may become entrapped at the entrance to the capillaries (Lipowsky, 2007).

In isolated *in situ* guinea pig hind limb preparation, elevated RBC aggregation had no significant effect on flow resistance with intact vascular control mechanisms. However, after inhibition of smooth muscle tone, perfusion with a series of increasing aggregating RBC suspensions, flow resistance first increased, then returned to control and finally increased again (Yalcin, et al., 2004). These results also support the complex effect of RBC aggregation on hemodynamics and the effect of exhausted vascular compensation (which is present in CHD).
Red blood cell deformability

RBC deformability has a decreasing trend, being significantly lower in non-significant vessel disease compared to patients with no vessel disease. Pfafferott, et al. (1999) reported no significant difference of RBC filterability.

RBC deformability is the primary determinant of flow resistance in blood vessels with dimensions similar to the RBC size. Lower RBC deformability negatively affects microcirculation by increasing the flow resistance in the capillaries. Again, the magnitude of this effect depends on the available vascular compensatory mechanisms. Baskurt, et al. (2004) reported that if RBCs were minimally hardened with glutaraldehyde, EI decreased by 16% and flow resistance increased by 78% in isolated-perfused rat hind limb. However, after the pharmacological elimination of vascular tone, the flow resistance increased by 250% for the same amount of deformability impairment. This suggest that in case of CHD, where the vascular compensatory mechanisms are limited, even a slight change in RBC deformability might have a great effect on myocardial microcirculation.

Other laboratory parameters

RBC count, Hgb concentration, MCV, MCH and MCHC were significantly increased in severe CAD. Increase of RBC count and MCV explains the same elevating trend of Hct. Increased Hgb concentration may be a counter mechanism against stenosis-caused low flow rate, in order to maintain oxygen delivery. In case of increased MCV, RBCs may require higher force to enter and to pass thought the capillaries. Elevated MCH and MCHC may increase intracellular viscosity, which can decrease RBC deformability (Cooke & Lim, 2007).

LDL levels were similar in each group. Though LDL is a CV risk factor and expected to be elevated in CAD patients, the much higher use of statins in CAD groups counteracts. The decreasing HDL levels were also expected in CAD.
**Study limitations**

This is a cross-sectional study; therefore inference on the relationship between the measured variables may be speculative. According to the latest American (ACCF/AHA/ACP/AATS/PCNA/SCAI/STS, 2012) and European guidelines (The Task Force on the management of stable coronary artery disease of the European Society of Cardiology, 2013), coronary CT is indicated in medium pre-test probability of CAD [Fig. 3.5.], while coronary X-ray angiography is performed on high risk patients. In our study this resulted in a lower number of patients with severe CAD compared to our previous study where 65% of patient had multi-vessel disease. Due to these guidelines, much fewer multi-vessel CAD cases were expected therefore 40% area stenosis was chosen as a cut-off-point. Another important difference between our studies is the “distance” between control and severely ill groups. We did not have healthy controls, and the most severe cases are likely to be less severe compared to our earlier study. As a consequence, the differences in severity of CAD between the groups are lesser and significant differences are harder to be observed. Another possible reason for the scarcity of significant differences between CAD subgroups is the relatively poor discriminatory power of CT: this method is good at excluding CAD, but not as good in distinguishing high-grade from non-high grade stenosis.

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![Fig. 3.5. Non-invasive testing in patients with suspected stable CAD. Based on: 2013 ESC guidelines on the management of stable coronary artery disease.](image-url)
Conclusions

Our results indicate that both macro- and microrheological parameters are altered in CAD, therefore myocardial oxygen supply may be reduced at both macro- and microcirculatory levels, and may play a pathophysiological role in the deterioration of this disease.
Summary of new scientific results

Hemorheological effects of moderate red wine consumption

Our controlled study demonstrated beneficial effects of 3-week moderate red wine consumption on hemorheological parameters in healthy volunteers:

1. Three-week red wine intake decreases whole blood viscosity in healthy subject.
2. As a consequence, red wine consumption improves hematocrit per whole blood viscosity ratio, suggesting improved oxygen transport efficiency of blood even in healthy volunteers.
3. Moderate drinking of red wine lowers red blood cell aggregation.
4. Moderate consumption of red wine improves red blood cell deformability.

Hemorheological parameters in CT-detected coronary artery disease

Our study revealed that hemorheological parameters are altered in CT-detected coronary artery disease:

1. Hematocrit is higher in patients with coronary artery disease. The increase was already significant in the least severe group.
2. Whole blood viscosity is elevated in patients with severe coronary artery disease.
3. Red blood cell aggregation is increased in severe coronary artery disease.
4. Red blood cell deformability is lower in patients with coronary artery disease.
5. These findings support that in CT-detected coronary artery disease beyond the impaired hemodynamic factors (stenosis), hemorheological parameters are also negatively affected.
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| Oktatási művek                                                                      |
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| Oltalmi formák                                                                      |
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| Ismeretterjesztő művek                                                              |
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Idézők disszertációban, egyéb típusban | 0 | --- | 1 | 8

Megjegyzések:

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1 A hivatkozások a disszertáció és egyéb típusú idézők nélkül számolva. A disszertáció és egyéb típusú idézők összesítve a táblázat végén találhatók.

2 Teljes tudományos közlemény ebben az adatbázisban:
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4 Ide értve a teljes közlemények listájában nem szereplő publikációkat, a nem ismert lektoráltságú folyóiratokban megjelent műveket és minden olyan tudományos művet, ami a I.-IV. sorokban nem került összeszámlálásra.

5 A disszertációk és egyéb típusú idézők nélkül számolva (részletek)