Division of Invasive Cardiology, Department of Cardiology, Medical Faculty,
Albert Szent-Györgyi Clinical Center,
University of Szeged

NEW ASPECTS IN THE PATHOPHYSIOLOGY OF SEVERE AORTIC VALVE STENOSIS

Ferenc Tamás Nagy MD

PhD Thesis

Tutors:

Forster Tamás MD, PhD and Attila Nemes MD, PhD

2012

PUBLICATIONS RELATED TO THE DISSERTATION

I. Diehl P[*], **Nagy F**[*], Sossong V, Helbing T, Beyersdorf F, Olschewski M, Bode C, Moser M. Increased levels of circulating microparticles in patients with severe aortic valve stenosis. Thromb Haemost 2008; 99: 711-9. Impact factor: 3.803

II. **Nagy FT**, Sasi V, Ungi T, Zimmermann Z, Ungi I, Kalapos A, Forster T, Nemes A. Correlations between myocardium selective videodensitometric perfusion parameters and corrected TIMI frame count in patients with normal epicardial coronary arteries. Int J Cardiol 2012; 155: 498-501. Impact factor: 6.802

III. **Nagy FT**, Horvath T, Ungi T, Sasi V, Zimmermann Zs, Kalapos A, Forster T, Ungi I, Nemes A. Aortic valve stenosis is associated with reduction in myocardial perfusion as assessed by videodensitometry on coronary angiograms. [Az aortabillentyű szűkülete együtt jár a koronarográfiás felvételeken videodenzitometria során meghatározott miocardialis perfúzió csökkenésével]. Orv Hetil 2012; 153: 1256-1262. Journal without impact factor.

OTHER PUBLICATIONS

I. Timinszky G, Tirián L, **Nagy FT**, Tóth G, Perczel A, Kiss-László Z, Boros I, Clarke PR, Szabad J. The importin-beta P446L dominant-negative mutant protein loses RanGTP binding ability and blocks the formation of intact nuclear envelope. J Cell Sci 2002; 115: 1675-87. Impact factor: 6.954

II. Nemes A, Kalapos A, Sasi V, Ungi T, **Nagy FT**, Zimmermann Z, Forster T, Ungi I. Detection of perfusion abnormalities on coronary angiograms in hypertension by myocardium selective densitometric perfusion assessments. Int J Cardiol 2012; 157: 428-9. Impact factor: 6.802

*These authors contributed equally

1. INTRODUCTION

1.1. Aortic valve stenosis

Aortic valve stenosis (AVS), characterized by obstruction to the left ventricular out flow tract is the most common valvular disease and the third most prevalent form of cardiovascular disease in the western world after hypertension and coronary artery disease. Mechanisms of the progression of aortic valve stenosis are still partly unknown. Involvement of mononuclear cells and of chronic systemic inflammation akin to vascular atherosclerosis has been suggested in previous studies. Cardinal manifestations of AVS are exertional dyspnea, angina, syncope and ultimately heart failure usually presenting only in severe cases. Physiological alterations behind angina pectoris-like symptoms are generalized endothel dysfunction and an increment in left ventricular mass leading to increased oxygen consumption, decreased arteriole density, coronary diastolic filling impediment and subsequently subendocardial ischemia.

1.2. Cell derived microparticles

Microparticles (MPs) are small cell membrane vesicles that are shed from cells upon activation. MPs can be detected and classified by their particular set of surface antigens. Using these means, platelet, leukocyte and endothelial microparticles can be distinguished and attributed to their respective cell of origin. MPs have been associated with systemic and vascular inflammation, endothel cell activation, angiogenesis and procoagulant states in different studies. They are clearly important players in the pathophysiology of vascular diseases and may also be used as in-vivo markers for inflammation and vascular damage. The possible role of microparticles in the progression of AVS however has not been studied.

1.3. Computer-assisted, myocardium selective videodensitometry

Recently novel computer-assisted videodensitometric methods have been introduced for the quantitative assessment of the angiographic “blush” appearance in acute myocardial infarction (AMI). Alongside others we have characterized myocardial perfusion by the ratio of maximal density (G_{max}) and the time to reach maximum density (T_{max}) of the time–density curves (TDCs) in regions of interest on X-ray coronary angiograms. Sensitized by vessel masking technique, it supplies easily obtainable, objective and quantitative information on short term outcome in AMI. Potential use of this method for evaluating myocardial perfusion in the non-acute coronary syndrome patient population is however not well documented.

2. AIMS

1. To determine the level of microparticle release and systemic inflammation in patients with severe aortic valve stenosis and thereby to elucidate the role of microparticles in the origin and progression of the disease.
2. To establish the relation between our novel computer-assisted, myocardium selective videodensitometric method for regional myocardial perfusion assessment and the TIMI frame count method in patients with epicardial coronary arteries without significant stenosis.
3. To demonstrate myocardial perfusion abnormalities in patients with severe aortic valve stenosis without significant epicardial coronary artery stenosis by computer-assisted, myocardium selective videodensitometric measurement.

3. PATIENTS AND METHODS

3.1. Cell-derived microparticles in aortic valve stenosis

3.1.1 Patients

From May 2005 to November 2006, 22 patients with AVS were enrolled prospectively in our first study at the Department for Cardiology and Angiology, University Hospital Freiburg. As control we examined 18 individuals that were admitted to our department for chest pain of unknown origin and in which an acute coronary syndrome, vascular disease and other relevant medical conditions were excluded. We also excluded patients and patient controls with diseases in which an increased level of MPs is known. Standard blood analysis (blood cell count, plasma coagulation parameters, cardiac enzymes, C-reactive protein (CRP) was performed in all patients. Severe AVS was defined as an aortic valve surface of 1.0 cm^2 or less, according to the American College of Cardiology (ACC)/ American Heart Association (AHA) guidelines for valvular heart disease. All AVS patients had chronic degenerative AVS. Patients with congenital valve disease or a history for rheumatic AVS were excluded.

3.1.2. Flow cytometry

Venous blood was collected without application of a tourniquet into 5 ml containers to a final concentration of 0.1 mM citrate using a 21-gauge butterfly needle. Samples were centrifuged at 1,550 g for 20 minutes (min) at 20°C to obtain platelet-poor plasma (PPP). PPP aliquots

were incubated with the specific antibodies for 25 min in the dark before sterile filtered phosphate buffered saline (PBS) was added up to a total volume of 500 µm and microparticles were quantified by flow cytometry. Size calibration was performed with nonfluorescent polystyrene microspheres. MPs were distinguished from blood cells by their size of <1.5 µm and numbers are given in counts per min (cpm). Platelet microparticles (PMPs) were detected as CD31+/CD61+ particles <1.5 µm of size or as CD62P+/CD61+ particles <1.5 µm, termed CD62P+ throughout the article. Leukocyte microparticles (LMPs) were defined as CD11b+ particles smaller than 1.5 µm). Endothelial cell microparticles (EMPs) were defined as CD62E+ particles smaller than 1.5 µm originating from endothelial cell activation. To quantify monocyte activation, whole blood (100 µl) was incubated gently shaking in the dark with 10 µl anti-CD11b-PE. After suspension in 2 ml cell lysis solution, samples were incubated for 20 min to lyse erythrocytes before centrifugation with 500 g for 5 min. The supernatant was removed and after washing twice with 2 ml PBS (Mg2+Ca2+) analyzed by flow cytometry. Monocytes were defined as CD11b+ cells within the appropriate monocyte gate. For quantification of granulocyte activation, CD11b+ cells were gated to include the granulocyte population. Activated leukocytes were quantified in fluorescence histograms, and medians were used for further statistical analysis as mean fluorescence intensity (MFI). Monocyte-PMP conjugates were assessed by detection of particles that were positive for both CD45 and for CD62P after gating the monocyte population in forward/sideward scatter. To detect conjugates between activated monocytes and PMPs CD11b antibody was used instead of CD45 antibody and samples were processed as described above.

3.1.3. Flow chamber experiments

To mimic different levels of shear stress *in vitro* we used the Glycotech flow chamber with gasket C. Flow was induced using a Harvard apparatus syringe pump. Flow rates were adjusted as described to achieve shear forces of 0.5 dynes/cm^2 and 15 dynes/cm^2. Citrated whole blood was obtained and subjected to the respective shear forces prior to analysis of MPs using our standard protocol.

3.1.4. Plasma levels of soluble P-selectin and interleukin-6 (IL-6)

Blood concentrations of soluble P-selectin and IL-6 were determined by commercial enzyme-linked immunosorbent assay (ELISA) kits from patient plasma that was obtained by centrifugation of citrated blood directly after collection and was stored until assaying at –80°C.

3.1.5. Statistical analysis

Data are presented as absolute and relative frequencies for categorical variables and as means with standard deviations for continuous variables. Correlations between selected variables were estimated by Spearman's rank correlation coefficient. Data of AVS and control patients was compared by means of Fisher's exact test for categorical variables and by the Wilcoxon rank test for continuous variables. All tests were two-sided and used a significance level of 5%. Data were analyzed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3.2. Evaluation of the relationship between computer assisted, myocardium selective videodensitometry and TIMI frame count

3.2.1. Patients

The second study comprised 43 patients with chest pain of unknown origin who had undergone elective coronary angiography with a negative result (<40% intraluminal epicardial coronary artery diameter stenosis) at the Division of Invasive Cardiology, Department of Cardiology, Medical Faculty, Albert Szent-Györgyi Clinical Center, University of Szeged . Patients with acute coronary syndrome, valve disease and left ventricular dysfunction were excluded from this study.

3.2.2. Technical aspects of coronary angiography and videodensitometric analysis

Angiograms for videodensitometric analysis were recorded on an Innova 2000™ system, in a way that phase matched digital subtraction angiography (DSA) can be performed on them. This required the following criteria: (1) motion of patient or table should be avoided; (2) patient should hold breath for the time of recording; (3) one contrast-free heart cycle should be recorded before injection of contrast material; (4) field of view is to be set to contain the whole supplied area of the vessel of interest. Standardized projections were chosen to minimize the superimposition of coronary arteries, veins and the aorta with the myocardium of interest. Constant quantity of nonionic contrast material (6ml) was injected for all angiograms by an automatic injector at a rate of 3 cc/sec in order to standardize the density of angiograms. Time-density curves were recorded in polygonal regions of interest (ROI) regarding the coronary artery of interest, selected by a cardiologist experienced in the analysis of coronary angiograms. Phase-matched digital subtraction angiographic images were used with standard image acquisition parameters. Visible vessels were automatically masked from the regions of measurements to eliminate their effect in the densitometric

signal. Time-density curves were analyzed to obtain Gmax/Tmax values indicative of myocardial perfusion.

3.2.3. TIMI frame count

Determination of frame counts was carried out by the method described previously by Gibson *et al.* The first frame was defined by a column of contrast extending across > 70% of the arterial lumen with ante grade motion. The last frame counted is that in which contrast enters (but not necessarily fills) a distal landmark. Left anterior descending (LAD)-TFC was corrected by a factor (1.7) to take account for longer distance to the TIMI landmark to gain corrected TIMI frame count (cTFC). In addition, a conversion factor of 2 was used to adjust for frame rate of 15 frame/sec used in our laboratory compared to the 30 frame/sec acquisition speed used in the original cine angiographic studies.

3.2.4. Statistical methods

Data are reported as means ± standard deviation. Data analyses were performed with statistical software Medcalc (Medcalc 11.3, Mariakerke, Belgium). $P < 0.05$ was considered to be statistically significant. Correlations of G_{max}/T_{max} with cTFC were assessed by Spearman's rank correlation coefficient.

3.3. Myocardial perfusion abnormalities in aortic valve stenosis as assessed by computer assisted, myocardium selective videodensitometry

3.3.1. Patients

The third study comprised 20 patients with AVS submitted to the Division of Invasive Cardiology, Department of Cardiology, Medical Faculty, Albert Szent-Györgyi Clinical Center, University of Szeged for preoperative evaluation before aortic valve replacement surgery. Patients with significant coronary artery disease (>70% intraluminal epicardial coronary artery diameter stenosis) were excluded from the study. 30 patients submitted for coronary angiography because of chest pain of unknown origin without significant coronary artery disease served as the control group. The control group was matched in regards to age, gender and ischemic heart disease risk factors.

3.3.2. Technical aspects of coronary angiography and videodensitometric analysis

Technical aspects of coronary angiography and videodensitometric analysis are described in section 3.2.2. In addition to single vessel related G_{max}/T_{max} values we introduced mean

G_{max}/T_{max} (average of single vessel related G_{max}/T_{max} values) to describe global myocardial perfusion.

3.3.3. Statistical analysis

Data are presented as absolute and relative frequencies for categorical variables and as means with standard deviations for continuous variables. Data of AVS and control patients were compared by means of chi square test for categorical variables and by the Student's T test for continuous variables. In case of the LAD related G_{max}/T_{max} Welch's test was used because of unequal variance. All tests were two-sided. $P < 0.05$ was considered to be statistically significant. Statistical software applied was Medcalc (Medcalc 11.3, Mariakerke, Belgium).

4. RESULTS

4.1. Cell derived microparticles in aortic valve stenosis

4.1.1. Clinical parameters

AVS patients and patient controls were similar in terms of age (AVS: 71.4 ±11.8 vs. (patient control (PC): 67.3 ±9.3 years), gender distribution (female male ratio: AVS: 13:9 vs. PC: 9.9), cardiovascular risk factors, blood cell counts, CRP level, cholesterol and body mass index. Notably, there was no significant difference in antiplatelet medication.

4.1.2. Echocardiographic parameters

The mean of the aortic valve surface of AVS patients was 0.65 ±0.16 cm², resulting in an elevated transvalvular gradient of 73 ± 28 mmHg and increased blood velocity (Vmax) of 4.1 ±1 m/s.

4.1.3. Platelet microparticles

The number of total PMPs (CD31+/CD61+) was significantly higher in AVS patients compared to patient controls (AVS: 868 ±600 cpm vs. PC: 504 ±230 cpm, p =0.046). In patients with AVS CD62P+ PMPs were also significantly increased (AVS: 76.0 ±44.5 cpm vs. PC: 47.8 ±21.8 cpm, p =0.028). No significant differences in platelet counts between both groups were observed (AVS: 237 ±70 1/nl vs. PC: 235 ±69 1/nl, p =0.92). PMP activation increased with augmenting shear stress in our patients.

4.1.4. Flow chamber experiments

As we have demonstrated shear stress-induced PMP generation in AVS patients we sought to corroborate these data by in-vitro experiments. Therefore we subjected whole blood from volunteers (n=4) to various levels of shear stress in a flow chamber and analyzed the generation of PMPs. In these experiments increasing shear forces significantly induced increasing amounts of PMPs, indicating that shear stress is a trigger for PMP release.

4.1.5. Leukocyte microparticles (LMPs)

Patients with AVS had significantly more LMPs compared to patient controls (AVS: 31.6 ±18.5 cpm vs. PC: 19.4 ±9.7 cpm, p =0.014).The number of leukocytes in the peripheral blood was similar in both groups (AVS: 6.63 ±2.00/nl vs. PC: 7.09 ±1.85/nl, p =0.34).

4.1.6. Leukocyte activation

Activated monocytes were increased in AVS patients compared to patient controls (AVS: 902 ±331 MFI vs. PC: 676 ±189 MFI; p =0.042). Accordingly, there was a trend towards increased numbers of activated granulocytes in AVS patients (AVS: 437 ±216 MFI vs. PC: 339 ±82 MFI; p =0.062).

4.1.7. Endothelial microparticles

The amount of CD62E+ EMPs was elevated in AVS patients compared to patient controls (AVS: 19.7 ±12.3 cpm vs. PC: 10.4 ±6.8 cpm, p =0.008), indicating endothelial cell activation in AVS patients. Most interestingly the extent of monocyte activation correlates with the number of CD62E+ EMPs supporting the hypothesis that monocyte activation contributes to activation of endothelial cells in AVS patients.

4.1.8. PMP-monocyte conjugates

The number of total PMP-monocyte complexes was significantly higher in AVS patients compared to controls. Using an activation specific antibody (CD11b) we found that almost all PMP-monocyte complexes contained activated monocytes.

4.1.9. Blood levels of soluble P-selectin and IL-6

We found that plasma P-selectin levels and IL-6 levels were not significantly different between AVS patients and patient controls with a trend to higher concentrations in AVS patients.

4.2. Evaluation of the relationship between computer-assisted, myocardium selective videodensitometry and TIMI frame count

4.2.1. Clinical parameters

During the present study 124 coronary arteries of 43 patients were analyzed for corrected TFC and G_{max}/T_{max} values. Because of anatomical variations 3 out of 43 left circumflex coronary arteries (CX) and 2 out of 43 right coronary arteries (RC) were not evaluated. G_{max}/T_{max} values measured for the respective arteries were LAD (2.85 ±1.56 1/sec), CX (2.63 ±1.36 1/sec) and RC (2.39 ±0.95 1/sec). Corrected TFC values measured for LAD (25.6 ±12.1 frames), CX (25.8 ±9.7 frames) and RC (26.0 ±9.9 frames) were comparable to previous data published concerning non-infarct related arteries**.**

4.2.2. Correlations

Low to moderate significant correlations were found between corrected TFC of respective arteries and LAD -G_{max}/T_{max} ($r = -0.57$, $p < 0.01$), CX -G_{max}/T_{max} ($r = -0.33$, $p < 0.05$) and RC -G_{max}/T_{max} ($r = -0.41$, $p < 0.01$).

4.3. Myocardial perfusion abnormalities in aortic valve stenosis as assessed by computer assisted, myocardium selective videodensitometry

4.3.1. Clinical parameters

During the third study we performed computer-assisted, myocardium selective videodensitometric analysis to obtain G_{max}/T_{max} values indicative of myocardial perfusion in 144 coronary arteries of 20 AS and 30 control patients. Because of anatomical variations 3 out of 20 right coronary arteries in the AVS group and 2 out of 30 right coronaries as well as 1 out of 30 circumflex arteries in the control group were not evaluated. In regards to clinical and demographic data AVS patients and the control group was similar except for age as the patients in the AVS group patients were significantly older.

4.3.2. Echocardiographic parameters

Peak transvalvular gradient was 84.2 ±32.5 mmHg and mean transvalvular gradient was 47.4 ±15.2 mmHg as measured by transthoracic echocardiography reflecting the severity of disease in the AVS group.

4.3.3 Computer-assisted, myocardium selective videodensitometry

In the AVS group G_{max}/T_{max} values regarding respective vessels were: LAD-G_{max}/T_{max} =2.78 ± 1.03 1/sec, RC–G_{max}/T_{max} =3.11 ± 1.45 1/sec, CX-G_{max}/T_{max} =2.00 ± 1.13 1/sec). In the control group these values were: LAD-G_{max}/T_{max} =3.44 ± 1.73 1/sec, RC-G_{max}/T_{max} =3.85 ± 15 1/sec, CX-G_{max}/T_{max} =2.83 ± 1.04 1/sec. Mean G_{max}/T_{max} describing global myocardial perfusion was 2.55 ± 1.02 1/sec in the AVS group and 3.39 ± 1.09 1/sec in the control group. G_{max}/T_{max} values indicative of myocardial perfusion were significantly lower in the AVS group then in the control group regarding the CX perfusion territory (p =0.011) and global myocardial perfusion (p =0.0088). Regarding the left anterior descending artery (p =0.0975) and right coronary artery perfusion territories (p =0.0682) we found a non- significant trend towards lower perfusion in the AVS group.

5. DISSCUSSION

5.1. Cell derived microparticles in aortic valve stenosis

The mechanisms of the progression of aortic valve stenosis are partly unknown. The involvement of mononuclear cells and of chronic systemic inflammation has been suggested by analysis of pathological specimens. In the first study reported in this thesis we hypothesized that shear stress caused by the constricted aortic orifice contributes to systemic proinflammation by activation of circulating blood cells and thereby generation of microparticles. Using flow cytometry we analyzed 22 patients with severe aortic valve stenosis (AVS) and 18 patient controls for the generation of circulating microparticles from platelet- (PMPs: CD31+/CD61+ or CD62P+), leukocyte- (LMPs: CD11b+) and endothelial cell (EMPs: CD62E+) origin. Apart from the constricted valve orifice groups were similar. PMPs were increased in AVS patients and their number correlated with valvular shear stress. Monocytes were activated in AVS patients, an observation that was also reflected by increased numbers of LMPs and by the detection of PMP-monocyte conjugates. Furthermore, EMPs reflecting the activation of endothelial cells but also conferring systemic inflammatory activity were increased in AVS patients and correlated with the number of activated monocytes. In conclusion, we showed that AVS is accompanied by increased levels of microparticles and that shear stress can induce the formation of microparticles. Based on our results and histologic findings of other investigators the speculation that shear stress related to aortic valve stenosis induces a vicious circle including the generation of PMPs, the

subsequent activation of monocytes and LMPs and finally the activation of endothelial cells contributing to the progress of aortic valve stenosis appears to be justified.

5.2. Myocardial perfusion abnormalities in severe aortic valve stenosis

5.2.1. Evaluation of the relationship between computer-assisted, myocardium selective videodensitometry and TIMI frame count

Recently alongside others we have introduced a novel computer-assisted videodensitometric method for the quantitative assessment of the angiographic "blush" appearance in AMI. We have characterized myocardial perfusion by the ratio of maximal density and the time to reach maximum density of the time–density curves in regions of interest on X-ray coronary angiograms. Although well documented in AMI, literature on possibility of interpretation in non-acute coronary syndrome settings is limited. In the second study comprising this thesis we set out to establish the potential use of this method in non-ACS patients by evaluating its relationship to the previous golden standard angiographic method of TFC in patients free of significant coronary artery disease. Correlations could be demonstrated between computerized videodensitometric myocardial blush parameters and quantitative coronary angiographic TFCs. Of further note is that stronger correlations were found regarding to LAD compared to RC or CX. Possible explanation for this phenomenon could be coronary anatomy variability, especially concerning the dominance of RC or CX which renders standardized ROI selection. The absence of stronger general correlation in relation to TFC could be explained by the TFC's indirect nature regarding myocardial perfusion, highlighted by its poorer comparable predictive value as previously described in AMI. For further validation of this method additional studies are warranted in comparison with established non-angiography-based imaging modalities preferably in specific cardiovascular diseases with microvascular dysfunction.

5.2.2 Myocardial perfusion abnormalities in aortic valve stenosis as assessed by computer assisted, myocardium selective videodensitometry

The third study presented in the dissertation was performed to further verify myocardial perfusion abnormalities present in severe AVS patients. For this purpose we measured G_{max}/T_{max} values indicative of myocardial perfusion by computer-assisted, myocardium selective videodensitometry in 20 AVS patients and 30 control patients. Significant epicardial

stenosis was ruled out during coronary angiography in both groups. We hypothesized that pathophysiological alterations led by an increment in left ventricular mass and generalized microvascular dysfunction would lead to a slower and less intense penetration of contrast material as quantitatively measured by $G_{max}/T_{max.}$ Indeed mean G_{max}/T_{max} values indicative of global myocardial perfusion were significantly lower in the AVS group than in the control group. The observed large standard deviation and only non-significant tendencies in regards to LAD and RCA related G_{max}/T_{max} must draw our attention to the limitations of perfusion assessment at rest in non-ACS patients. Namely coronary blood flow, myocardial perfusion and microvascular resistance at rest is mainly determined by hemodynamic parameter driven autoregulation mechanisms, and subject to variations according to gender and age. In future studies it would seem prudent to uncouple autoregulation of myocardial circulation through achievement of maximal hyperemia by intracoronary or intravenous adenosin infusion. G_{max}/T_{max} values at hyperemia by themself or as a ratio of baseline G_{max}/T_{max} akin to CFR may prove to more specific and sensitive marker of microvascular dysfunction. In conclusion reduced G_{max}/T_{max} values indicative of impaired myocardial perfusion as measured by videodensitometry on coronary angiograms could be demonstrated in AS compared to normal controls.

5.3. LIMITATIONS

As acknowledged throughout this thesis although data presented here taken together with cited prior observations give strong circumstantial evidence that shear stress generated microparticle interaction with blood cells and endothelium is pivotal in the progression of aortic valve stenosis, they fall short of conclusively establishing a cause-effect relationship. In addition to our cross-sectional study, more definitive support for an active role of microparticles in the pathogenesis of aortic valve stenosis will require a prospective longitudinal study correlating microparticle-cell interaction with progression of aortic valve stenosis.

Computer-assisted, myocardium selective videodensitometry is a novel method under constant development as technological advances permit. Major technical issues yet to be tackled include problems concerning the standardized selection of static ROI areas. Large ROI areas can result in variable results due to inhomogeneous distribution of contrast material. On the other hand, small static ROI area selection is difficult for the whole image

sequence because of the cyclic motion of myocardium caused by the heart beating. Further problems inherent to nonautomated ROI selection is the possibility of intra- and interobserver variability. These factors in part may be accountable for variable average Gmax/Tmax values reported for similar control groups discussed in this thesis. Of further note is the commercial unavailability of our software and those of others which renders wide spread use and unbiased, multicenter testing.

6. CONCLUSIONS (NEW OBSERVATIONS)

- Severe AVS is accompanied by increased levels of circulating microparticles and monocyte activation.
- Shear stress induces the formation of microparticles both *in vitro* and in AVS *in vivo*.
- Based on our results and histological findings of other investigators a novel hypothesis may be formed that shear stress related to AVS induces a vicious circle including the generation of platelet microparticles, the subsequent activation of monocytes and leukocyte microparticles and finally the activation of endothelial cells contributing to the progress of aortic valve stenosis
- Significant correlations exist between computer-assisted, myocardium selective videodensitometry-derived parameters and quantitative coronary angiographic TFCs in patients free of significant epicardial disease indicating possible usefulness of this method in the non-acute coronary syndrome patient population.
- Reduced Gmax/Tmax values indicative of impaired myocardial perfusion can be demonstrated by computer-assisted, myocardium selective videodensitometry on coronary angiograms in severe AVS compared to normal controls.

7. ACKNOWLEDGEMENT

First of all I would like to thank my wife Zsuzsa and my sons Balázs and Zalán for their support and patience during the long and tiresome road needed to complete this thesis.

I would also like to thank my mother, father and uncle not only for their support but also for serving as excellent role models with their pre-eminent academic careers.

I am most grateful to Professor Martin Moser for providing me with an excellent and stimulating atmosphere for doing research at the University of Freiburg and for willing me on in the troubled beginning period when I thought microparticle isolation is not possible.

I would like to express my deepest gratitude to Dr. Imre Ungi who is not only my mentor in the field of interventional cardiology but together with his son Dr. Tamás Ungi the founder of the computer-assisted myocardium selective videodensitometric method for assessment of myocardial perfusion discussed in the second part of this thesis.

I would never have been able to finish my dissertation without the guidance of my supervisors Dr. Attila Nemes and Professor Tamás Forster who never let me lose sight of my final goal.

Last but not least I would like to thank my colleagues here at the University of Szeged who not only helped in recruiting patients for my study but also substituted me in every day clinical practice during my year abroad.