THE INVASIVE AND NON-INVASIVE EXAMINATION OF CIRCULATORY SYSTEM FOCUSED ON CHANGES IN DYNAMICALLY PERFUSED CORONARY CAPILLARY SYSTEM

PhD thesis

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I. Introduction

The human body works like a system that contains many subsystems, all of them acting and counteracting to each other. To better understand them, one should measure at a specific time point or in case of more complex situation, the cycles of the living system. One of the most interesting and complex part of the human body is the circulatory system that can be observed by simple methods, such as palpate the pulse, or in a more sophisticated way, such as magnetic resonance (MR) measurements. One of the most important aspects of the system is to describe the normal function or to analyze the reaction to stress situation. Circulatory system can be well described with three independent parameters; such as pressure (P), volume (V) and time (t). During the daily practice we call them blood pressure (RR, wich is equal to P), heart rate (HR, wich is the reciprocal rate of time - heart beat over the one minute period). In critical condition we would like to measure the cardic output (CO) or cardiac index (CI) (CO equal flow, practically V/t). Many of our examination tools are not able to measure these three independent parameters at the same time by a noninvasive way, some times even very difficult to measure the correct data by invasively also. The control of the human body is very sophisticated and one of the key element of the circulatory system regulation is the endothelial cells. Vascular endothelium participates in various important physiologic processes, such as hormone synthesis and degradation, prostaglandin synthesis, release and uptake, lipid processing and xenobiotic metabolism. These functions of the different organs may be changed even when morphological or clinical signs of endothelial dysfunction are absent. Healthy human vascular endothel metabolize circulating substances and exhibit evidence of altered endothelial function during various conditions, including cardiopulmonary bypass and pulmonary hypertension, permeability edema, ARDS, etc.. Endothelial ACE function has been reported that various types of injury (bleomycin, hyperoxia, chest irradiation, phorbol ester acetate, cardiolplegic solution, etc.,) can affect the interaction of these enzymes with their substrates. Endothelial cell injury also occurs in a number of chronic illnesses, notably autoimmune diseases, atherosclerosis and diabetes. The vascular pathology of diabetes mellitus has long been categorized into macroangiopathy and microangiopathy. Diabetic macroangiopathy is very similar to non-diabetic atherosclerotic lesions. The lesions, however, are more diffuse, more severe and more prominent in peripheral arteries. The pathogenesis of vascular disease is multifactorial, endothelial functional and structural injury however, appear to be an early event. Endothelial structural alterations, and adhesion of

leukocytes and platelets onto the cell surface were apparent in diabetic rabbit aortas, as early as two weeks after alloxan injection. Impaired endothelium-dependent relaxation has been demonstrated in several species and vessel types.

Endothelium-bound ACE is an ideal probe for estimating the function and/or size of the perfused capillary bed, *in vivo,*

1. because ACE is uniformly distributed throughout the luminal endothelial plasma membrane surface;
2. because endothelium-bound ACE is an ectoenzyme, and this allows the repeated use of exogenously administered circulating substrates with negligible tissue accumulation of substrate or product;
3. by virtue of the continuously increasing ratio of luminal wall surface area to luminal volume, as vessel diameter decreases, the highest hydrolytic activity is concentrated in capillaries, with less than 10% of total activity being attributed to vessels of diameter >20μm.

This thesis contains three animal experiments to investigate and analyze coronary circulation and myocardium perfusion in different conditions. The noninvasive part of the thesis contains three human studies, because recent developments in computer technology allowed us to build more sophisticated devices (such as the impedance cardiograph – ICG, or the newly developed arteriograph) to measure not only in resting but during excercise conditions the above mentioned parameters.

II. Animal experiments

1. AgII and ET-1 interaction in dog model

Experimental protocol

To analyze the interaction of the two most aggressive vasoconstrictor agents such as AgII (angiotensine II) and ET-1 (endothelin-1), the following experiment was carried out:

Sixteen mongrel dogs of either sex weighing 10-23 kg were anesthetized by i.v. injection of pentobarbital sodium (30-35 mg/kg). Artificial positive pressure ventilation was started through an endotracheal tube by room air. Thoracotomy was performed between the fourth and fifth ribs and the heart was suspended in a pericardial cradle. An electromagnetic flow probe (Statham SP 2201) was placed around the LAD to measure blood flow and a small

indwelling catheter (o.d. < 0.8 mm) was inserted into the LAD for administration of drugs. Unipolar electrodes were sewn onto the epicardial surface of both ventricles and atria for electrical stimulation and recording of electrocardiograms. Standard bipolar and unipolar surface ECG leads were continuously recorded. All parameters were recorded on a 12-channel direct writing recorder (Schwartzer). Before the experiments the animals were heparinized (500 mg/kg, i.v.).

Results (see figure 1.)

Bolus injections of AgII (7.8×10^{-13} to 3.9×10^{-11} M) and ET-1 (10^{-12} to 10^{-9} M) induced a dose-dependent decrease in coronary blood flow (CBF) ([DELTA]CBF_{max} -82± 10% for AgII and -91 ± 8% for ET-1). Simultaneous AgII and ET-1 boluses had slightly smaller effects on CBF than the calculated additive figure. Five-minute infusions of AgII (10^{-12} to 10^{-10} M/min) and ET-1 (5×10^{-12} to 2×10^{-10} M/min) induced a slight decrease in CBF ([DELTA]CBF_{max} -12± 9% for AgII and -19 ± 9% for ET). Background ET-1 or AgII infusions did not alter the dose-response curve of the other drug. Simultaneous Ag II and ET-1 infusions at different rates (10^{-12} to 10^{-10} M/min for AgII and 5×10^{-12} to 2×10^{-10} M/min for ET-1) over 5 min had similar effects on CBF as the calculated additive figure ([DELTA]CBF_{max} -35 ± 17% for the joint administration of the highest doses). Bolus administration of ET-1 and AgII resulted in transient coronary vasoconstriction. Similar amounts given over a 5-min period had only slight effects on coronary vascular tone. Bolus injections of the two drugs in combination resulted in a less degree of vasoconstriction than the calculated additive value from individual injections.

2. Perfused coronary surface measurements in dog

Experimental protocol

Experiments were performed using seven mongrel dogs (five males and two females), weighing 18.6–28.6 kg (mean 22.2 ± 0.8 kg).

After the surgical procedure was completed, eight measurements were performed, at approximately 15-min intervals. Two transpulmonary measurements of ACE activity and perfused capillary surface area were carried out before (P1) and after (P2) the six transcoronary measurements. Three pair transcoronary measurements were performed, at 50% flow reduction (E1), at 75% flow reduction (E2), and after ligation (E3) of the first diagonal branch (distal to the flow probe) of the LAD. Each measurement took place 5 min after

reduction of flow; coronary flow was returned to normal immediately after the E1 and E2 measurements. Control (C1, C2, and C3) transcoronary measurements were carried out before each LAD flow reduction and served as baseline values for E1, E2, and E3 measurements, respectively. The transcoronary hydrolysis (v) of the specific ACE substrate, [3H]benzoyl-Phe-Ala-Pro, was estimated and the parameter Amax/Km (proportional to the size of the perfused CCSA) was calculated.

Results

By means of a ligature placed around the LAD, LAD blood flow was transiently reduced to 36.0 ± 4.1 (E1) and 17.4 ± 4.3% (E2) of
control; in a separate maneuver the first diagonal branch of the LAD was ligated to achieve 40.0 ± 6.7% (E3) of control flow. The v values remained unchanged at around 0.7 for E1, E2, and E3 determinations, suggesting unaltered substrate transit time through the coronary capillary bed. Amax/Km values decreased to 36±5, 17±4, and 47±10% of control for E1, E2, and E3 determinations, respectively, reflecting a flow-proportional decrease in CCSA. Values of the transpulmonary measures of v and Amax/Km performed at the beginning and end of the protocol were unchanged.

3. Cardiac MRI for the diagnosis of ischemic events in the heart

Experimental protocol

ECG-gated, MR image sets were collected in transiently LAD-occluded dogs (animal model was discussed earlier). Either Gd(ABE-DTTA) (N = 6) or Gd(DTPA) (N = 6) was injected, and imaging was continued for 30 minutes of ischemia and 40 minutes of reperfusion. The contrast agent (CA)-induced MRI signal intensity enhancement (SIE) and contrast were monitored. Microspheres measured myocardial perfusion (MP) was used to verify areas of ischemia and reperfusion.

Results

SIEs of 86% ± 3% and 97% ± 3% in nonischemic, and 25% ± 5% and 29% ± 8% in ischemic regions were found within three minutes of onset of ischemia with Gd-(ABE-DTTA) and Gd(DTPA), respectively. For the rest of the 30 minutes of ischemia, with Gd(ABE-DTTA) SIE of 60% ± 3% and 25% ± 5% persisted in the nonischemic and ischemic regions, respectively. With

Gd(DTPA), however, SIE in the nonischemic areas decreased rapidly after the first three minutes of ischemia, while SIE in the ischemic areas increased, abolishing contrast. (see fig.3.).

III. Human studies

1. Impedance cardiography (ICG) measurements

Study design

12 healthy volunteers (negative anamnesis, ECG and normal hemodynamic state) were involved in study. Basic and calculated hemodynamic parameters (Z_0 – basic impedance, VET – ventricular ejection time, PEP – preejection period, SV- stroke volume, CO – cardiac output, CI – cardiac index, SVR – systemic vascular resistance) were recorded by ICG during resting and they were recorded 4hour and 24hour later. We also compared the parameters recorded simultaneously by ICG and spiroergometry .

Another 12 healthy volunteers (negative anamnesis, ECG and normal hemodynamic state) and 12 hyperacid patients (documented with pentagastrin test and similarly negative cardiovascular anamnesis, ECG and normal hemodynamic state) were involved in the discrimination study. Hemodynamic state of the subjects were recorded by ICG during resting – stability, stress (cold pressor test, right hand was immersed to ice water) and during recovery phase. The relative changes were calculated and the data for the normal and hyperacid subjects were analyzed by unpaired t-test, finally PRIMA method as a multivariate statistical analysis was used.

Results

Based on the stability and reproducibility measurements, the Z_0 parameter is the best to analyze and/or compare data intraindividually . During the exercise period the correlation between ICG and spiroergometry was good ($r = 0.81$ fig. 4.). During the CPT, in a short time period a higher increase in HR (heart rate) was observed in normal subjects whereas the same time Z_0, RR_{sys}, RR_{dia}, VET were higher in hyperacid group. As we expected after the learning phase of PRIMA could well separate the two different groups of volunteers (see fig. 5.) .

2. Arteriograph validation study

Study design

We validated a newly developed simple, user independent and fast oscillometric, portable device (Arteriograph) measuring augmentation index (Aix) and aortic pulse wave velocity (PWV-ao) simultaneously against invasive, intra-arterial measurements of the mentioned parameters. Our comparative study was performed on patients who underwent routine coronarography for diagnostic purposes. In 10 cases we measured the brachial Aix (Aix-br) with intra-brachial cannula and Arteriograph, and in 13 cases the aortic Aix (Aix-ao) with intra-aortic catheter and the Aix-br with Arteriograph simultaneously on identical pulses. In 27 cases the invasively and non-invasively measured PWV-ao was compared. Out of the 27, in 11 cases we used 2 catheters (inserted from radial and femoral artery) positioned to the aortic root and to the aortic bifurcation and the transit time of the pulse wave was measured on identical heart cycles. In the remnant cases the PWV-ao was determined with one catheter with pull back from the aortic root to the bifurcation and the transit time was measured using ECG gating.

Results (see fig. 6.,7.8.)

The correlation between invasively and Arteriograph measured Aix-brachial/brachial, Aix-aortic/brachial and PWV-Ao were 0,92 ($p<0,001$), 0,92 ($p<0,001$) and 0,82 ($p<0,001$) respectively. With Bland-Altman plots the differences were within 2SD in all of the compared parameters.

IV. Summary and thesis

1. Using a canine model and based on the intracoronary administration of two vasoactive agents such as ET-1 and Ang II we conclude that they do not interact in the coronary vascular bed. One interesting feature was that joint infusion of the two drugs decreased coronary vascular tone in some experiments more than the calculated additive value of separate experiments. However, this was observed only in a small number of cases, and we were unable to characterize this subgroup of animals in any way that might explain the disproportional effect of the drugs. Our findings do not exclude the possibility that ET-1 and Ang II can induce fatal vasoconstriction, but rather indicate that subthreshold intracoronary doses are insufficient to induce such an effect.

2. Endothelium-bound ACE is an ideal probe for estimating the size of the perfused capillary bed, in vivo, (a) because ACE is uniformly distributed throughout the luminal endothelial plasma membrane surface (b) because endothelium-bound ACE is an ectoenzyme, and this allows the repeated use of exogenously administered circulating substrates with negligible tissue accumulation of substrate or product.

3. Earlier published results suggested that decreased coronary flow reduces surface area by capillary derecruitment. In our study, the regional Amax/Km is analogous to the PS product. There were no significant differences in substrate utilization between two successive control determinations (at similar flow), indicating that capillary enzyme activity was unaltered during the experiment. Our data demonstrated a linear correlation between coronary flow and the size of the perfused capillary surface area (Amax/Km).

4. Our MRI study demonstrates that Gd(ABE-DTTA)-enhanced ceMRI, which shows persistent effects during ischemia and dissipation of contrast in a myocardial perfusion-dependent manner during reperfusion, enables the detection of ischemic events in the heart.

5. Based on the relative changes of the ICG data during the stability measurements we conclude that the basic, measured parameters more stabile than the calculated parameters.

6. Analyzing the results of the newly developed combined CPT-ICG-PRIMA method (which practically a statistically powered stress analysis) we could separate different group of patient based on the responder organs (analyzing of the heart reaction could separate the gastrointestinal vagal tone or reaction).

7. The new oscillometric Arteriograph device can measure accurately the central (aortic) and peripheral (brachial) Aix and aortic PWV, which means that by noninvasivaly could be measured the marker of rigidity of vascular system.

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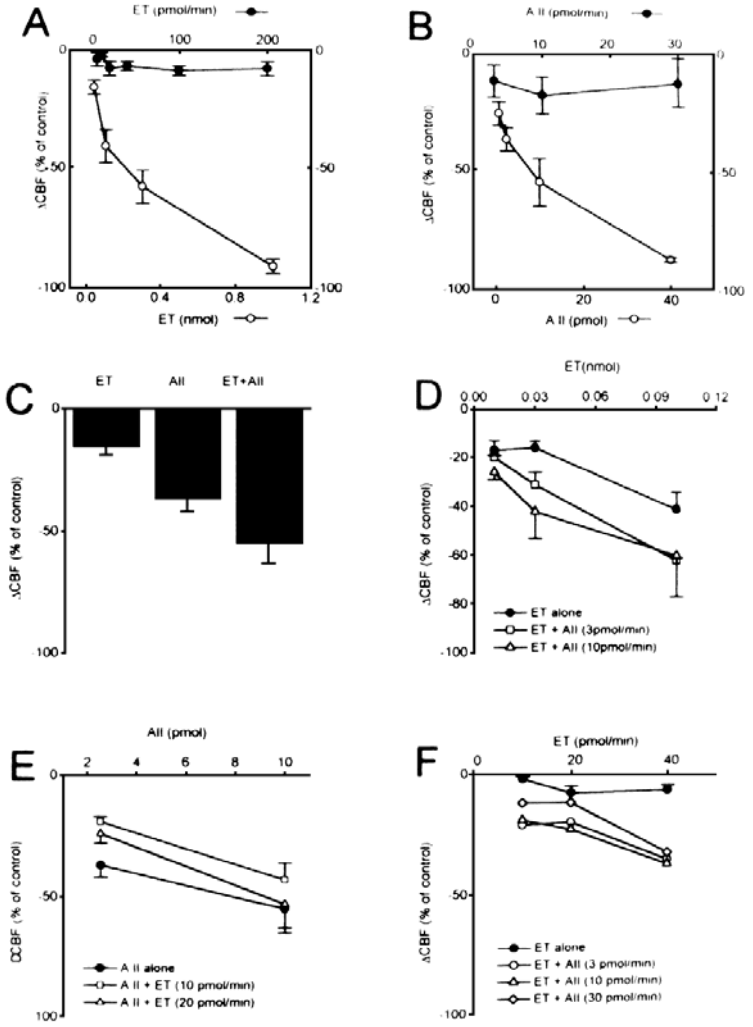
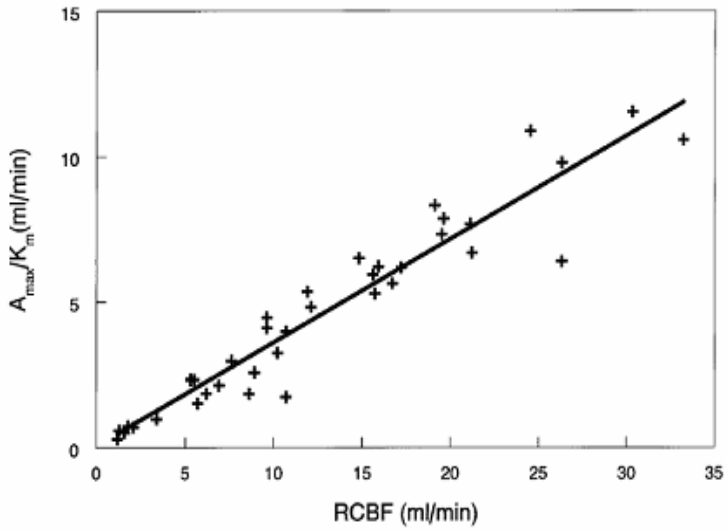
VI. Figures



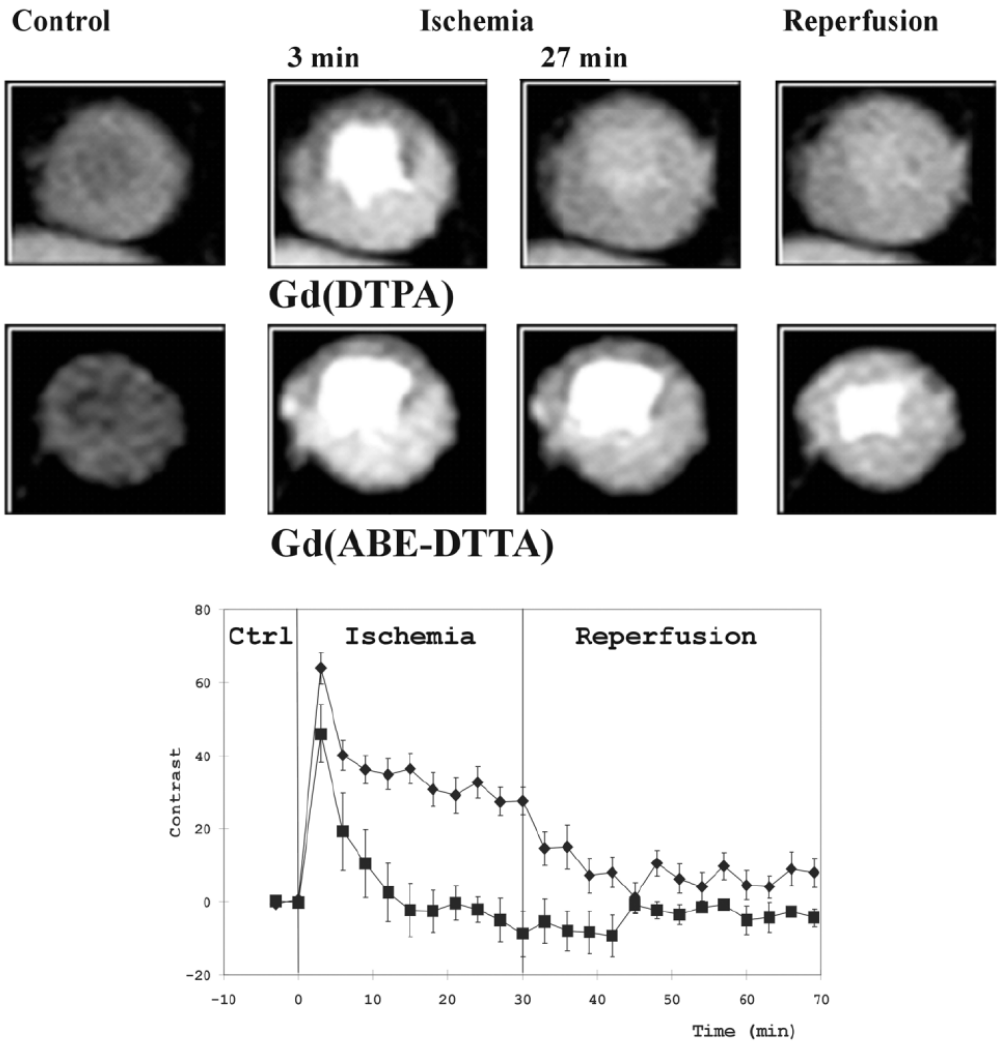
Effect of intracoronary administration of ET-1 and AgII (AII) on coronary blood flow in dogs. Drugs were administered into the left anterior descending coronary artery (LAD) via an indwelling catheter, either as boluses or as a 5-min infusions. The effect of each drug was also tested superimposed on background subthreshold infusions of the other drug. A: Effect of ET-1 boluses and infusions alone. B: Effect of AgII boluses and infusions alone. C: Joint effects of ET-1 and AgII boluses resulting in additive values. D: Effect of ET-1 boluses alone or with various background infusions of AgII. E: Effect of AgII boluses alone or with background ET-1 infusions. F: Effect of ET-1 infusions alone or with various AgII background infusions. Presented data are sown as mean±SEM. SEM bars are omitted from F panel because of wide variations.

Figure 1.



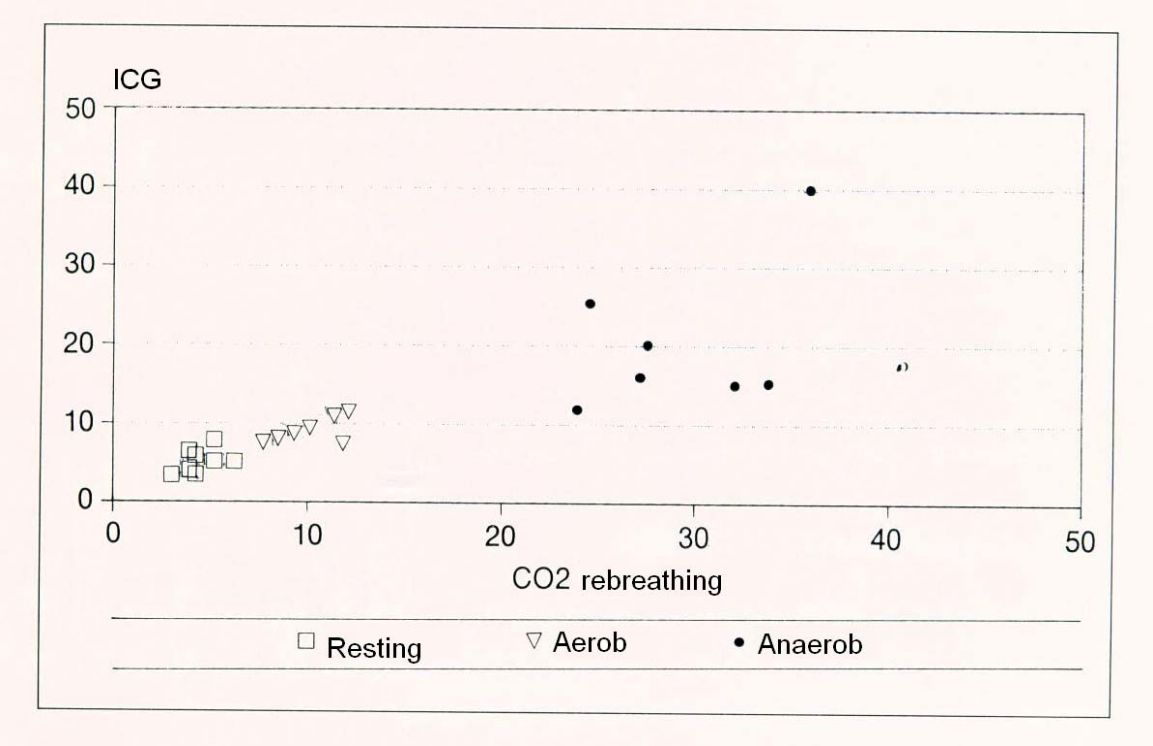
Correlation between regional coronary blood flow (RCBF) and size of the perfused coronary capillary surface area (A_{max}/K_m). Data combined from all maneuvers of reduced coronary flow ($N = 38$). $y = 0.36x + 0.066$; $r = 0.91$; $P < 0.001$.

Figure 2.



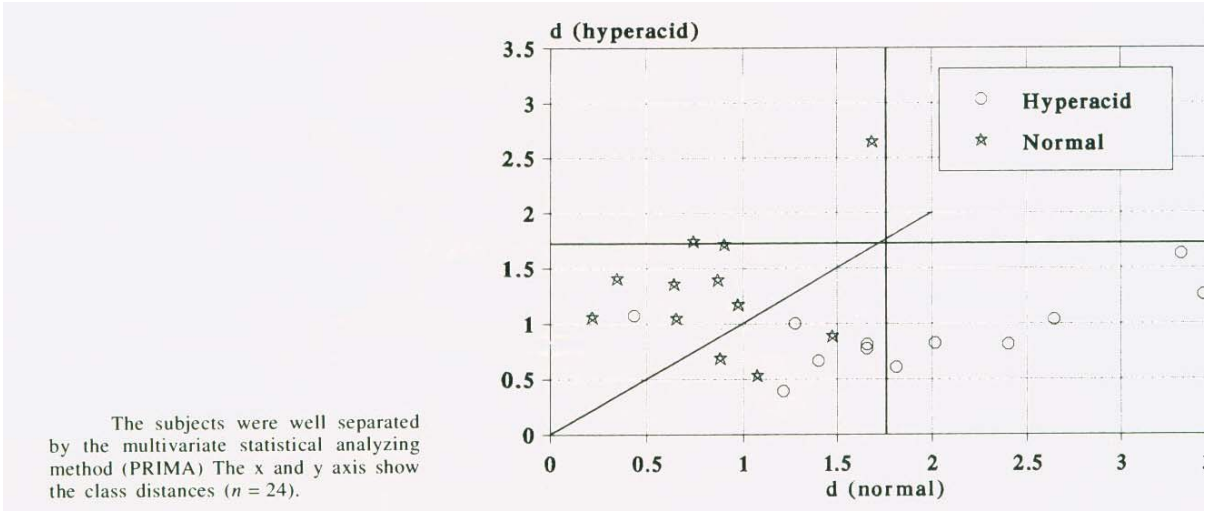
Time dependence of myocardial contrast in dogs a: Representative images from the control period, at 3 and 27 minutes of ischaemia and at 3 minutes of reperfusion are shown with either Gd(DTA) or Gd(ABE-DTTA). b: Contrast vs. time with Gd(ABE-DTTA) (N=6, diamonds) or with Gd(DTPA) (N=6, squares)

Figure 3.



Cardiac output measurements during resting and exercise conditions (aerobic and anaerobic) The correlation coefficient during exercise is $r = 0.81$.

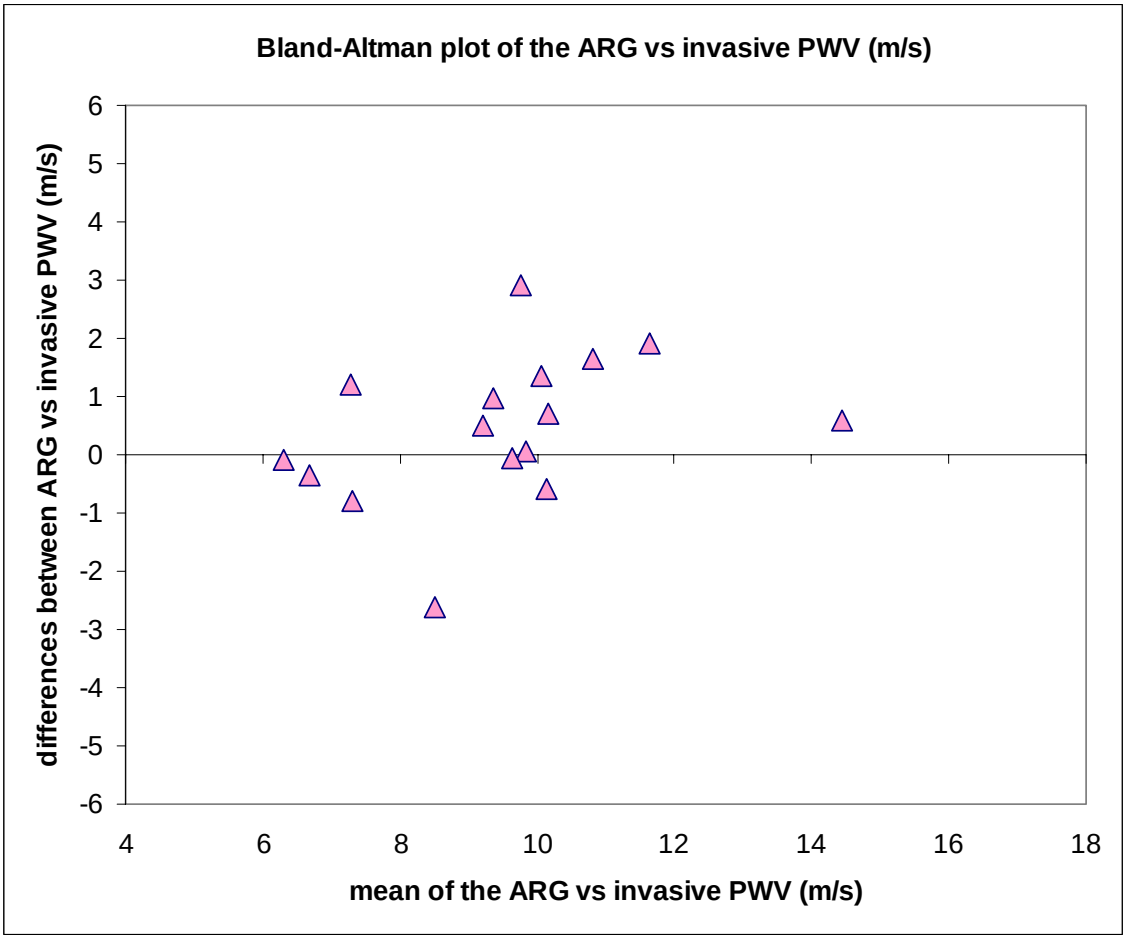
Figure 4.



The subjects were well separated by the multivariate statistical analyzing method (PRIMA) The x and y axis show the class distances ($n = 24$).

CPT-ICG-PRIMA method to separate different kind of patients CPT based hemodynamic changes depend on vascular reactions.

Figure 5.



Pulse wave velocity data measured by arteriograph non-invasively and at the same time invasively using diagnostic Judkins catheters.

Figure 6.

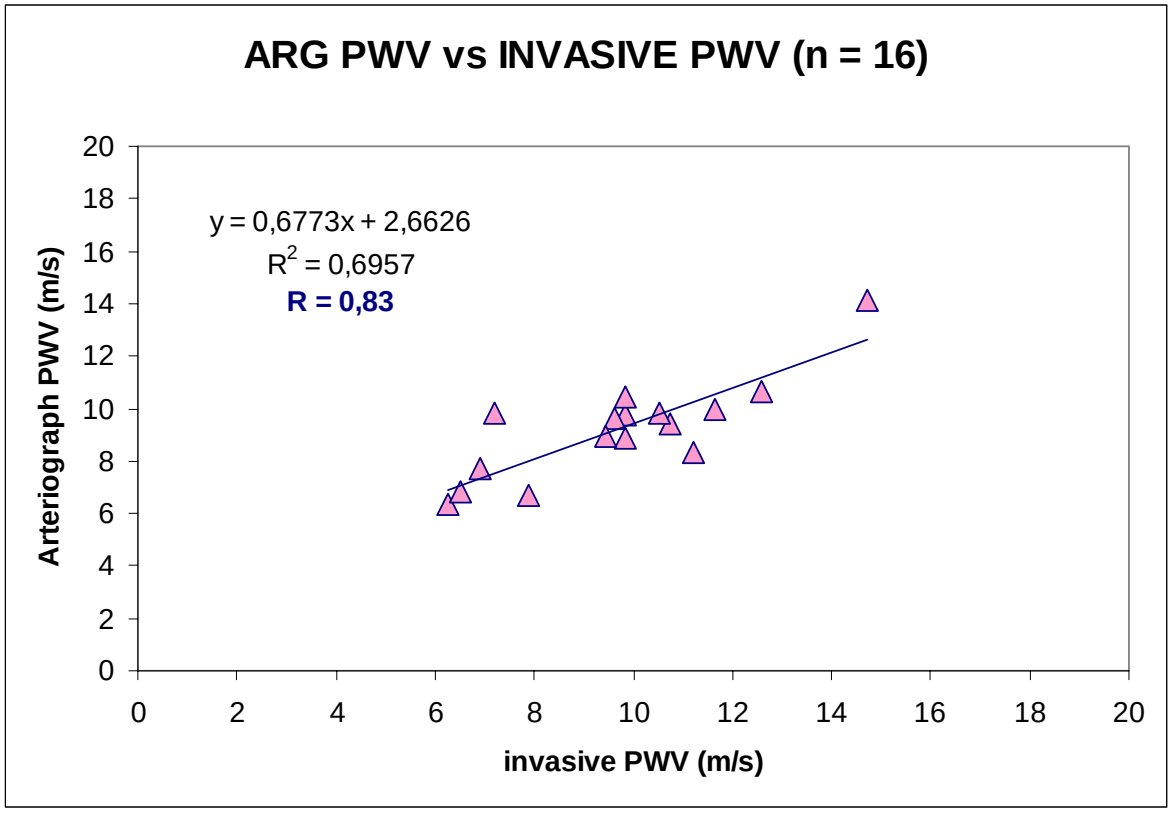

ARG PWV vs INVASIVE PWV (n = 16)
y = 0,6773x + 2,6626
R2 = 0,6957
R = 0,83
Arteriograph PWV (m/s)
invasive PWV (m/s)
0
2
4
6
8
10
12
14
16
18
20

Figure 7.

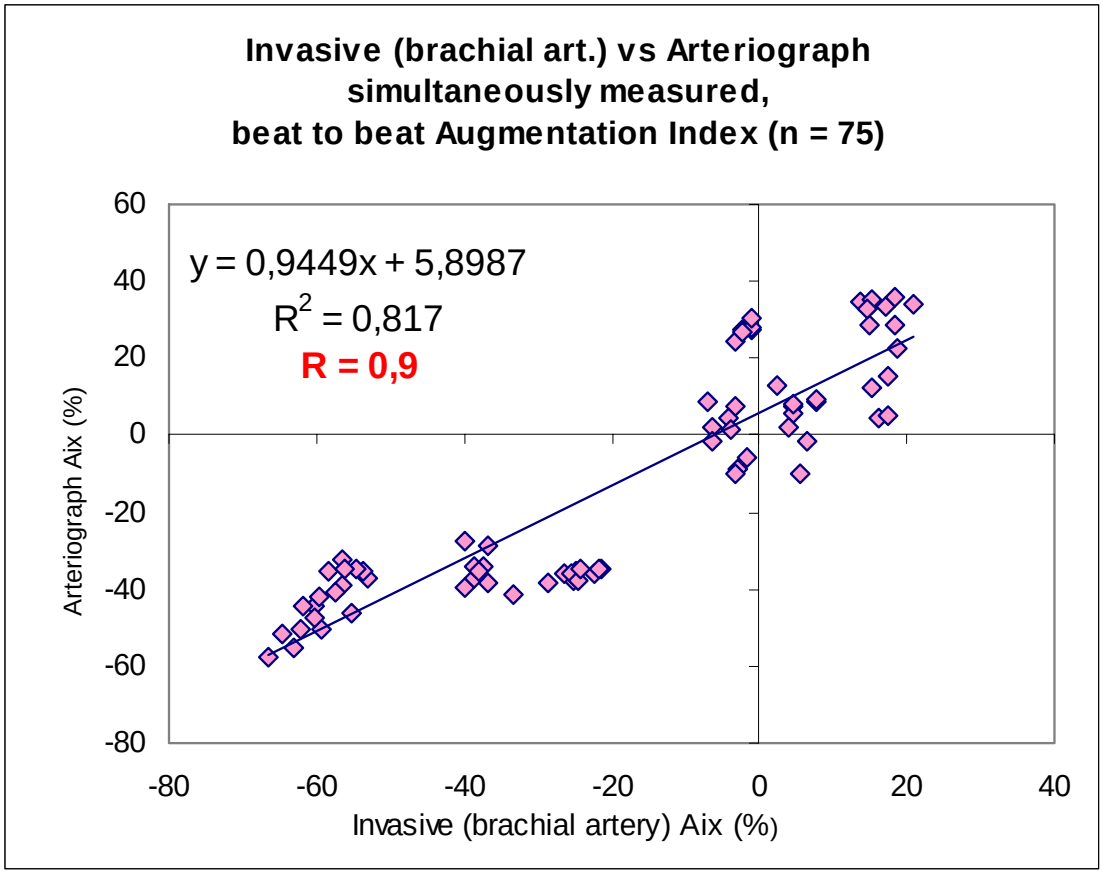

Invasive (brachial art.) vs Arteriograph
simultaneously measured,
beat to beat Augmentation Index (n = 75)
y = 0,9449x + 5,8987
R2 = 0,817
R = 0,9
Arteriograph Aix (%)
Invasive (brachial artery) Aix (%)
60
40
20
0
-20
-40
-60
-80
-80
-60
-40
-20
0
20
40

Figure 8.