Ph.D. Theses

CARDIOVASCULAR ALTERATIONS FOLLOWING CORONARY ARTERY BYPASS GRAFTING PROCEDURES

1. Studies on Causes of Early Postoperative Death
2. Infection by Chlamydia pneumoniae in Coronary Artery Bypass Grafts

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4. Summary

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1. INTRODUCTION

Coronary artery bypass grafting (CABG) belongs to the state-of-the-art of the surgery of atherosclerosis, one of the most important diseases in the western world. In all our investigations we sought primarily what kind of gross and micromorphologic cardiovascular alterations develop following CABG procedures, and whether these have pathophysiological and clinical relevances?

Two main fields were observed. (1) On one hand, we examined entities of the early postoperative period following CABG (0-30 days after surgery) to give a clinico-pathologic evaluation on causes of death during this period. (2) In the second part of our activities our attention was focused on chronic alterations following CABG, mainly on possible relations to the widely investigated infective agent of the vasculature, Chlamydia pneumoniae (C. pneumoniae).

(1) In view of the inconsistent use of terms in previous related reports, the unelaborated criteria they apply and so, the lack of their comparability, we decided in order to meet these shortages to put forward a clear-cut system of criteria for the evaluation of causes of death and by this means to give basis for the comparability of similar studies in the future. In order to further try our system of criteria, we attempted to use it with two related open heart surgery cohorts (valvular operations and combined interventions /valvular + CABG operations/) to see if a better evaluation can be achieved, and whether the results so obtained correspond to the general pathophysiological setting of the surgical intervention under scrutiny.

(2) In the second part of our activities it was our objective to study if accelerated long-term occlusion of coronary artery bypass grafts is related to infection by C. pneumoniae, and if conclusions on the pathophysiology of the infective process can be drawn from morphologic alterations? To control our observations and conclusions, similar investigations were performed on various venous structures from different patient groups and coronary artery samples from young adults, and special attention was given if any of these results mutually confirmed each other, so implying a more general importance of the alterations observed.
2. STUDIES ON CAUSES OF EARLY POSTOPERATIVE DEATH

"THESIS"

Thesis ? 1. Clinico-pathological studies on death causes following coronary artery bypass grafting (CABG) have rarely been reported. Definitions of basic terms were a prerequisite not only for the sound interpretation of our own material but also for the benefit of comparison between future series.

Background: After Favaloro, and Green et al reported in 1968 on improving impaired blood flow to myocardium in severe coronary artery disease by use of vascular bridging, coronary artery bypass grafting (CABG) has gained wide acceptance. Morphological studies on changes after CABG were mainly done on venous grafts. It was noted that various causes may lead to decreased graft flow, e.g. (1) placement of a disproportionate graft on a small coronary artery, (2) distal anastomosis on an arteriosclerotic stenosed coronary artery segment, (3) kinking of a too long graft, (4) a graft trimmed too short causing stretching and flattening of the grafting vessel, (5) spasm of a saphenous graft, (6) damage of the graft-endothelium and thrombosis as a result of the operational manipulation, etc. There are only few clinicopathological studies on patients dying after CABG.

Material and Methods: We investigated the autopsy material of the Institute of Pathology, Bethesda Hospital, Duisburg (Germany) that resulted from the operational material of the Cardiac Centre of Duisburg. Between January 1992 and June 1995, 5749 CABG procedures were performed in this Cardiac Centre; the mortality rate was 3.8%. For a retrospective clinicopathological analysis 32 cases were amenable.

A systematic detailed macroscopic investigation of the heart specimens was carried out followed by an evaluation with conventional histomorphology. Special attention was paid to the state and alterations of the native coronary artery system, the grafts and the supplied myocardium. This was followed by a clinicopathological consensus-evaluation, through which the mechanism of death corresponding both to clinical and morphological findings was sought.

Results and Discussion: By means of the above methodology a clinicopathologically based evaluation was put down on the cause of death in every single case. Setting up a system from these experiences a table containing criteria on causes of death was created. In short, two major groups of causes of death were defined:


(1) cardiac; and 
(2) non-cardiac causes of death.

Cardiac causes of death (1) were subclassified into two further groups:

(1a) Causes intrinsic to the heart that could be on one hand
    (1a1) death due to severe coronary artery disease,
    and
    (1a2) death due to chronic heart failure,

on the other.

(1b) Causes not intrinsic to the heart, i.e. surgery related complications.

Amongst cardiac causes (1) death was regarded as due to severe coronary artery disease (1a1) in any case where no evidence existed for a surgery-related complication or a non-cardiac cause of death and

- a circumscribed early postoperative acute myocardial infarction developed in a myocardial area supplied by a coronary artery with distal or diffuse coronary artery disease where complete revascularisation was not feasible, or

- despite central coronary artery disease an incomplete revascularisation was done because (a) coronary artery disease was preoperatively undetected by angiography or (b) coronary artery configuration was intraoperatively evaluated as technically inoperable (e.g. technically inaccessible coronary artery anatomy/intramural position, vessel vulnerability, etc.); otherwise incorrect assumption of a diffuse type coronary disease with resignation to revascularize) or (c) excessive calcification in the ascending aorta (so-called porcelain aorta) allowed no placement of a proximal anastomosis with a circumscribed early postoperative acute myocardial infarction in the related area of a diseased but not grafted vessel, and/or

- operation was performed with a preoperative acute myocardial infarction.

Chronic heart failure (1a2) was taken as cause of death in any case where

- without coronary artery disease or with completely revascularised central coronary artery disease histologically extensive myocardial damage could be evidenced typically coupled by a severely depressed left ventricular function preoperatively, or

- the patient had a cor bovinum (heart weight >800g) and

- no evidence existed for surgery-related complications or a non-cardiac cause of death.
Surgical complication (1b) was taken as cause of death in any case with acute myocardial infarction that occurred in the early postoperative period in a patient with relatively good preoperative left ventricular function and
- the acute myocardial infarction was found in an area supplied by a grafted coronary vessel with a significant (>75%) acute stenosis or occlusion (e.g. through thrombosis or vessel dissection) either in the proximity of the anastomosis, or in the grafting vessel or in the native coronary artery distal to the anastomosis and/or
- the acute myocardial infarction developed in an area supplied by a revascularized coronary artery showing significant primary (coronarosclerotic) stenosis at the anastomotic site and/or
- evidence exists for multiple acute thrombi of unknown origin in revascularized and/or non-revascularized coronary arteries without evidence of technically inoperable coronary artery disease or a non-cardiac cause of death and/or
- the acute myocardial infarction is extensive affecting several myocardial areas without non-revascularized stenoses/occlusions of corresponding coronary arteries or stenosed/occluded grafts in a patient with an early postoperative low-output-syndrome, without evidence of death due to a non-cardiac cause

Non-cardiac causes (2) were briefly defined as 'any disease of extracardiac origin limiting vital functions'.

In the related literature reviewed by us the use of ill-defined, often non-systematic and non-corresponding terms for causes of death was experienced, that hindered interpretation of diagnoses and comparison of different series. The definitions proposed by us give a clear meaning to our expressions on the one hand, and - in case of their future application - may help comparison of mortality data of different cardiac centres. This gains special importance if we regard, that postmortem examination - despite a drastic decrease of autopsy rate through the last decades in the western world - is still acknowledged as one of the most specific indicators of clinical activity with special respect to clinical diagnoses, management of patient care, judgement and technique of surgical practice, and so, it represents an invaluable means for quality assurance and quality control.
Thesis 2. Early postoperative death following CABG is very often linked to surgery related complications. Similarly, severe coronary artery disease indicating the major pathological alteration leading to surgery, causes death in a comparable proportion. Our system of criteria on causes of death helps mortality evaluations in other types of open heart surgery, too, and the results thus obtained fit into the general settings defined by the corresponding diseases.

**Background, Material and Methods:** See under Thesis 1.

**Results and Discussion:** According to the criteria quoted under Thesis 1, in the group of patients treated with CABG (n=32) death was due to surgical complications in 43% and severe coronary artery disease in 41%. Cases with death due to chronic heart failure (13%) and non-cardiac cause (3%) were substantially rarer. The following technical problems were detected: (a) thrombosis of graft or anastomosis; (b) dissection of graft or native coronary artery; (c) kinking of a too long graft; (d) a graft trimmed too short with stretching and flattening, cutting into the subepicardial fat; (e) the suture being pulled too tight at the distal anastomosis distorting and stenosing the anastomotic configuration; (f) placement of the distal anastomosis on an arteriosclerotically stenosed coronary artery segment with subsequent decreased flow; (g) thrombotic occlusion of an anastomosis initiated by a piece of adjacent myocardium lifted by the suture into the anastomotic lumen.

Early postoperative death following valvular operations (n=9) was caused by surgical complications in 33%, chronic heart failure in 33%, non-cardiac cause in 22% and severe coronary artery disease in 11%.

Analysis of cases after combined interventions (n=7) showed a remarkably high percentage of early postoperative death caused by surgical complications (57%). Chronic heart failure, severe coronary artery disease and non-cardiac cause were represented substantially rarer and in an equal frequency (14% each).

Though the limited number of cases in the analyzed groups allowed for no statistical evaluation, yet the tendency could be observed that surgical complications ranked first among death causes in all three intervention groups. The importance of the main cardiovascular alteration leading to the given type of surgical intervention is shown by the fact that these were the second most important causes of death in each group: severe coronary artery disease in the CABG-group; chronic heart failure in the valvular operations group, the latter
corresponding to an already preoperatively restricted pump capacity secondary to long-term valve dysfunction. Interestingly, severe coronary artery disease and chronic heart failure was represented in the combined intervention group quite rarely and in an equal rate referring to the excessive mortality importance of the enhanced operative burden on one hand, and to the balanced role of the doubled heart pathology (i.e. coexistence both of coronary artery and valve disease) on the other.

3. Infection by Chlamydia pneumoniae in coronary artery bypass grafts

Background: Chlamydia pneumoniae has been identified since 1989 as a separate species and an agent frequently causing respiratory diseases, such as bronchitis, pharyngitis, pneumonia, even asthma. Its prevalence in human individuals in various communities is high. The pathogenetic role of C. pneumoniae in atherosclerosis was suggested primarily on the basis of seroepidemiologic studies, the organism has been detected in sclerotic lesions in various parts of the vasculature by several methods, such as immunohistochemistry, polymerase chain reaction (PCR) and cell-culture. The question whether C pneumoniae represents an innocent bystander or posseses an active role in atherogenesis remains to date undecided despite extensive human and experimental research in the field. The possible role of C pneumoniae in late coronary artery bypass failure has been so far investigated in a few studies only.

Material and Methods: Coronary artery bypass grafts with long-term failure were investigated in order to detect their infection by C. pneumoniae. The collection of material was organized in collaboration with three leading cardiosurgical centres in Budapest (Hungary), where 53 redo coronary artery bypass grafting procedures were performed between May 1998 and May 1999. Twenty-one of these were amenable to our study. A total of 34 grafts with long-term occlusion and 28 new grafts were collected to be submitted to
an evaluation by routine histomorphology, immunohistochemistry and polymerase chain reaction (PCR).

During conventional histologic evaluation, graft occlusive lesions were characterized according to the classification of the American Heart Association (AHA) for atherosclerotic lesions. For the examination of the immunohistochemical reactions by the light microscope, vascular wall layers were divided into two compartments: intima-media and adventitia. The intima and the media were considered as one common compartment, as these were often involved together in the sclerosing process so much so that differentiation of the two was not possible. Infection was graded separately in both compartments according to the number of positively stained cells found in the total transectional area of a given vessel: grade 0 (infection-free) – no positive cells; grade 1 (mild infection) – positive cells between 1-10; grade 2 (moderate infection) - positive cells between 11-50; grade 3 (severe infection) - positive cells over 50. In one instance electron microscopic examination was performed for the direct visualization of the bacterium on the tissue of a vessel sample previously shown by immunohistochemistry to harbour a grade 3 infection. PCR-detection was done in two independent laboratories by two different methods. Blood samples were collected for serologic examinations.

Results and Discussion: Infection by C. pneumoniae was detectable in all 21 patients with immunohistochemistry. In vascular samples (n=62) immunohistochemical evidence of infection at least in one of the mural compartments and at least in a mild degree was seen in 97% of occluded grafts and in 96% of new grafts. The electron microscopic examination performed on one of the vessel samples to control specificity of the immunohistochemical reactions could despite previous formalin fixation, paraffin embedding and thus dissatisfactory tissue preservation demonstrate the presence of pear-shaped particles with a large periplasmic space and dimensions of 0.5?m corresponding to the elementary bodies of C. pneumoniae. PCR detection in the two independent laboratories demonstrated positive results in 11 of 61 cases (19%) examined by the PCR-RFLP (restriction fragment length polymorphism) protocol on frozen material, and in 14 of 42 cases (33%) examined by the TETR-PCR (touchdown enzyme time release) method on deparaffinized material. On 41 samples both of the PCR protocols could be performed, giving identical (either positive or negative) results in 30 cases (73%); in 6 instances (10%) the result was correspondingly
positive. One or the other of the two PCR protocols gave positive result in 19/62 cases (31%).

Results of our investigations confirmed the data widely documented in the literature reporting on a varying detectability of *C. pneumoniae* largely depending upon the method used. Amongst the established detection methods immunohistochemistry ranked most efficient in our experience, too, leaving the among other circumstances far more sensitive PCR to be a distant secondary. Discordance between the two different PCR protocols was nearly 30%.

The prevalence of the bacterium is substantially higher in our series than in other reports, which may be explained by our extensive tissue sampling, the performance of serial sections (62 vessel samples - 220 immunohistochemical slides - averaging 3.54 immunohistochemical slides / vessel) and that we chose immunohistochemistry for our main detection method. No immunohistochemistry was done in the few series reported so far in the literature upon redo CABG-patients, despite the fact that this is acknowledgedly the most sensitive method to demonstrate *C. pneumoniae*. Furthermore, this may also imply that patients candidate for redo CABG represent a highly selected investigation material in whom the prevalence of *C. pneumoniae* may be higher than in members of other patient subgroups with symptomatic atherosclerosis.

Thesis 4. Adventitial presence of *C. pneumoniae* is especially frequent both in coronary grafts with long-term failure and in new grafts, which implies that chlamydial invasion happens in the first place from the peripheral, rather than from the luminal side of the vessel wall.

Background, Material and Methods: See under Thesis 3.

Results and Discussion: The intima-media of grafts with long-term occlusion frequently (82%) harboured infection as demonstrated by immunohistochemistry; infection-rate in the adventitia was even higher (97%). It is to be noted that of the total of 34 occluded grafts only one had an infection-free adventitia. New grafts (n=28) contained infection in the intima-media in 46% by immunohistochemistry; the rate of adventitial positivity was 96% (p<0,05). Prevalence of *C. pneumoniae* in all of the collected vascular material (n=62) was 66% in the intima-media (n=41) and 97% in the adventitia (n=60) (p<0,05). Main/remaining lumen endothelium contained *C. pneumoniae*
at the same time rather rarely: in only 9 of the total of 62 vessel samples (15%).

The high rate of adventitial infection by *C. pneumoniae* both in occluded and in new coronary grafts as contrasted by a rare detection of the bacterium in the main lumen endothelium suggests that chlamydial invasion of vascular tissues is more likely through peripheral small vessels (lymphatics) by means of infected macrophages as vectors than from the main lumen. This possibility has already been proposed in a few recent reports, and it is also supported by our statistical analyses. The number of collected samples allowed for statistical calculations only on venous grafts and it was found that both the mere presence of infection and the presence of severe (grade 3) infection was significantly more frequent in the occluded than in the new grafts, whereas no statistically significant difference could be demonstrated between these two vessel groups regarding the prevalence of adventitial infection.

**Thesis ? 5. Results of our investigations on infection characteristics of vessel wall compartments (intima-media vs. adventitia) suggest that in vascular infection by *C. pneumoniae* a certain persistent 'adventitial baseline infection' may play a role, from which infection of the intima-media develop according to local microenvironmental conditions.**

**Background, Material and Methods:** See under Thesis ? 3.

**Results and Discussion:** Through an accurate immunohistochemical-morphological evaluation of the vessel samples, division of the vessel wall into two compartments (intima-media vs. adventitia) and the definition of the degree of infection separately in these compartments, we were alerted to recognize that most grafts – irrespective whether occluded or new, venous or arterial and, furthermore, independently of the presence/absence or degree of infection in the intima-media – presented a surprisingly uniform mild-to-moderate infection in the adventitia. This makes the impression as if a permanent 'adventitial baseline infection' existed, from which infection of the intima-media would develop according to actual local microenvironmental conditions in varying degrees from vessel to vessel.

The above are completed by our partially preliminary observations made in two related series. The ‘adventitial baseline infection’ of vessels seems probable in several independent patient cohorts with different cardiovascular
pathologies (patients with cardiac arrhythmia and lower limb varicosity) and in heterogeneous populations (young adults dying for various reasons and subjects with sudden /natural and violent/ death). This may imply that it is a phenomenon of a more general importance suggesting that what here has been noted as an ‘adventitial baseline infection’, is indeed the local sign of a latent bacteremia of the diseased human individual. Some authors have previously reported that \textit{C. pneumoniae} is also present in organs other than the lung and the cardiovascular system, yet, it shows a predilectional affinity to cardiovascular tissues. Wide detectability of the bacterium seems to support the possibility of the above mentioned bacteremia.

4. SUMMARY

There are only few studies in the literature clinicopathologically analyzing early postoperative death following CABG procedures, and these apparently lacked clear-cut definitions for basic terms. In order to fill up this gap it was our objective to create such definitions for the benefit both of our own material and the comparability of future studies. This acquires special importance in view of the fact that post-mortem examination – and with it, clinicopathological evaluation – is acknowledgedly one of the most specific means to measure clinical work in hospital units and serve quality assurance and quality control. The evaluation system elaborated by us divides causes of death into two major groups (cardiac vs. non-cardiac causes) and characterizes them by means of four group-diagnoses (1./ severe coronary artery disease; 2./ chronic heart failure; 3./ surgical complications; 4./ non-cardiac causes). By using this system we found that leading cause of early postoperative death following CABG was some sort of complication directly related to the operational intervention. It is remarkable, however, that severe coronary artery disease representing the main cardiovascular pathology leading to surgery caused death in a similar proportion. We found furthermore, that this system of criteria helped mortality evaluations in other open heart surgery cohorts (valvular operations and combined interventions /valvular + CABG operations/) as well, and that the results obtained corresponded to the general pathophysiological settings of the diseases. So we observed the tendency that surgical complications were leading cause of death in all three open heart surgery cohorts. The importance of the main cardiovascular pathology leading to a given type of intervention was emphasized by the fact that these ranked second in the mortality list. So it turned out that the second most important cause of death was severe coronary artery disease in the CABG-group, and chronic heart failure in the valvular operations group. Severe
coronary artery disease and chronic heart failure was represented equally and quite rarely in the combined interventions group, which could refer to the excessive mortality importance of the enhanced operative burden on one hand, and to the balanced role of the doubled heart pathology (i.e. coexistence both of coronary artery and valve disease) on the other.

The presence of *C. pneumoniae* was found with immunohistochemistry very frequent both in chronically occluded and in new (venous and arterial) coronary bypass grafts in patients candidate for redo CABG. Adventitial prevalence of the bacterium proved to be especially frequent both in new grafts and in grafts with long-term occlusion, while it was only rarely detected in the endothelial lining of the main lumen, which implies that infection of vascular tissues is more likely from the peripheral than from the luminal side of the vessel wall. This was supported by our statistical analyses showing that infection in the intima-media of occluded grafts was significantly more frequent than infection in the intima-media of new grafts, yet, in the adventitia no such statistically significant difference could be demonstrated. Based on the analysis of characteristics of infection in the wall layers (intima-media vs. adventitia) we propose that a certain persistent ‘adventitial baseline infection’ may play a role in the infection of vascular structures by *C. pneumoniae*, from which infection of the intima-media develops according to local microenvironmental and/or other factors. This assumption is supported by the partly preliminary results of two of our further series showing that the existence of the ‘adventitial baseline infection’ may be probable in independent patient groups with various cardiovascular pathologies (patients with cardiac arrhythmia and lower limb varicosity) and in heterogeneous populations (young adults dying for various reasons and subjects with sudden/natural and violent/death). All this might show that this phenomenon is of a more general importance suggesting that what here has been noted as an ‘adventitial baseline infection’, is indeed the local sign of a latent bacteremia of the diseased human individual.

5. REFERENCES

Publications thematically related to the present thesis

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Textbook chapter


Abstracts


**Oral presentations**


15. Glasz T: Kardiovasculáris rizikófaktorok és jelentőségük, a megelozés, ill. kezelés jelentősége – a patológus szemével. *Továbbképző konferencia, Országos Alapellátási Intézet / Pécsi Tudományegyetem Családorvostani*

17. Kádár A, Illyés Gy, Glasz T: Development of atherosclerosis. IX. Slovenský angiologický kongres s medzinárodnou účast'ou (IX. Szlovák angiologický kongreszus nemzetközi részvétellel); Tátraotthon (Tatranské Zruby), Szlovákia 2001 október 10-12.

Publications not directly related to the present theses

Original articles


**Book**


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