Relation Between Coronary Artery Stenosis and Myocardial Purine Metabolism, Histology and Regional Function in Humans

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In 54 patients undergoing elective or emergency aorto-coronary bypass grafting, angiographic and electrocardiographic changes were studied. Five patients with unstable angina and five patients with evolving myocardial infarction were included. High energy phosphate metabolism and the histologic appearance of the myocardium were analyzed in transmural biopsy specimens acquired at the time of surgery.

In patients without anterior infarction on the electrocardiogram, severe stenosis of the left anterior descending coronary artery resulted in a reduction of anterior wall motion that was associated with a partial depletion of the adenylate pool. Mitochondrial function, however, remained intact: the adenosine diphosphate/adenosine triphosphate ratio, the energy charge and the creatine phosphate/adenosine triphosphate ratio were in the normal range. Histologic assessment demonstrated viable myocardium with a high incidence of atrophic cells.

In evolving myocardial infarction, 170 minutes of acute coronary artery obstruction resulted in anterior wall akinesia associated with a decrease of the sum of the adenylates to 52% and of creatine phosphate to 16% of their normal value (p < 0.05). The nucleosides accumulated; their major fraction (91%) was inosine. The adenosine diphosphate/adenosine triphosphate ratio increased from 0.14 ± 0.04 to 0.49 ± 0.20 (p < 0.01) and the energy charge decreased from 0.924 ± 0.021 to 0.660 ± 0.169 (p < 0.01). Ultrastructure examination revealed irreversible cell damage in at least the subendocardial layer.

These results suggest that the energetic base of reduced contractility due to severe coronary artery stenosis is different from that in acute coronary obstruction: the decrease in contractile function cannot be explained by a reduced oxidative phosphorylation but may be related to an impaired energy utilization at the myofibrillar level, in analogy with the postischemic reperfused stunned myocardium.

The effect of acute coronary artery occlusion on high energy phosphate metabolism has been extensively studied in experimental animals (1–5). Under these conditions a rapid degradation of adenine nucleotides has been demonstrated and a critical level of adenosine triphosphate (ATP) was proposed as the triggering mechanism for irreversible myocardial cell damage (1). Acute coronary artery obstruction in humans, however, is usually preceded by a significant degree of coronary artery stenosis and the influence of such a chronic, progressive stenosis on purine metabolism, myocardial function and cell viability has not been established. Recurrent episodes of regional ischemia associated with a severe degree of coronary stenosis may finally influence these variables. Indeed, recent experimental evidence suggests that intermittent myocardial ischemia induces persistent contractile dysfunction (6), changes in cell volume regulation (5) and depression of the adenine nucleotide pool (4,5) despite the prevention of cell death by adequate reflow.

We studied high energy phosphate metabolism and histology in the myocardium of patients with coronary artery disease at the time of surgery and related these findings to the degree of coronary stenosis and to regional wall motion as measured on the preoperative angiogram. Because the study group included patients with stable angina pectoris, unstable angina pectoris and evolving or healed myocardial infarction, a wide range of coronary stenosis progressing to complete occlusion was examined.

Of special interest are the observations on the rate of adenine nucleotide degradation in patients with evolving...
myocardial infarction because of possible discrepancies between experimental animals and human patients in terms of ischemia tolerance of the myocardium and time constraints for effective treatment after the onset of acute coronary artery obstruction.

Methods

Study patients. Fifty-four surgical patients with obstructive coronary artery disease were included in the study. The patients (6 women and 48 men) were 47 to 68 years old and all underwent aortocoronary bypass grafting. All patients had ventriculographic and coronary arteriographic examinations within 8 weeks before surgery. Five patients had unstable angina pectoris. Another five had coronary bypass surgery performed as an emergency procedure because of evolving myocardial infarction; four of these patients developed their acute infarction during cardiac catheterization or coronary angioplasty. The duration of occlusion of the left anterior descending coronary artery before revascularization was 170 ± 25 minutes.

Catheterization and angiography. In each patient angiograms of the left ventricle were obtained in the 30° right anterior oblique and the 45° left anterior oblique projection. Coronary angiograms were performed using the Sones technique. Ejection fraction was calculated and wall motion was measured from the frontal end-diastolic and end-systolic outlines of the left ventricle as described previously (7). The degree of stenosis in the left anterior descending coronary artery was determined by measuring the prestenotic, poststenotic and intrastenotic vessel diameter in both projections. The mean intraluminal pre- and poststenotic area (mm²) was calculated using the catheter diameter for calibration. The percent stenosis was calculated by expressing the intraluminal area at the narrowest site of the stenosis as a percent of the mean pre- and poststenotic area.

Patient groups. The patients were classified into groups according to the degree of stenosis of the left anterior descending coronary artery and electrocardiographic (ECG) signs of anterior infarction. Group I comprised 16 patients with 70 to 89% stenosis. Group II comprised 27 patients with 90 to 100% stenosis; of whom 5 had unstable angina. Only patients without electrocardiographic signs of previous anterior infarction were selected for groups I and II. Group III comprised five patients with evolving anterior infarction; acute occlusion of the left anterior descending coronary artery was confirmed during surgery in all five. Group IV comprised six patients with electrocardiographic signs of previous anterior infarction (healed infarction).

Myocardial biopsy. Two transmural needle biopsy specimens measuring 1.5 mm in diameter (Tru-cut biopsy needle, Travenol Laboratories) were obtained from the center of the area perfused by the left anterior descending coronary artery. These specimens were obtained when the patients were on cardiopulmonary bypass but before any of the distal or proximal coronary anastomoses were performed. Each specimen used for microscopic studies was divided into a subepicardial and subendocardial sample.

Biochemical analysis. The transmural biopsy specimens were immediately (within 3 seconds) cooled in liquid nitrogen and stored at -80°C. Adenosine triphosphate and creatine phosphate were determined by luminometry (LKB 1250; ATP luminescence kit, CLS-Boehringer). Nucleotides, nucleosides and the purine bases were separated using high pressure liquid chromatography in one single run (gradient on reversed phase C18 column) (8). Inorganic phosphate was measured using an automated malachite green method. Tissue contents are calculated as micromoles per gram dry weight.

Myocardial histology. The biopsy specimens were immersed in fixative containing 3% glutaraldehyde and 90 mmol/liter oxalate adjusted to pH 7.4 with normal potassium hydroxide. Further processing for light and electron microscopy was done as described previously (9).

In an attempt to quantify the degree of cell degeneration in the different layers of the myocardium, 100 myocardial cells that were sectioned through the nucleus were examined. This was done on semithick toluidine blue-stained sections of the subepicardial and subendocardial parts of the biopsy samples. The percent of normal cells was calculated (9).

Ultrastructural changes in the myocytes were analyzed in several electron micrographs that were taken at random for both areas (subepi- and subendocardial). No attempt was made to quantify the degree of subcellular myocardial damage.

Statistical methods. Biochemical, functional and histologic data are expressed as mean values ± standard deviation. For statistical analysis one way analysis of variance was performed between groups. Unpaired t test versus group I was performed when the F value was significant at the 0.05 level.

Results

Coronary artery stenosis and high energy phosphate metabolism (Table 1). Data on myocardial creatine phosphate, inorganic phosphate, nucleotides, nucleosides and the purine bases in the different groups are listed in Table 1. As coronary artery stenosis progressed, a loss of high energy phosphates was observed. With severe stenosis (group II), the sum of adenylates decreased to 73% of the value measured in patients with mild stenosis (group I). Adenosine triphosphate (ATP) content dropped to 74%. As long as the coronary artery was not acutely obstructed, purine catabolism was not associated with increased tissue content of nucleosides and purine bases (Fig. 1). However, when acute occlusion of the coronary artery developed (group III), the sum of adenylates dropped to 54% of the value found with mild stenosis. Then adeny late catabolism was reflected by
Table 1. Biochemical Data in 84 Patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodosis (%)</th>
<th>Patients</th>
<th>ATP (μmol/g DW)</th>
<th>ADP (μmol/g DW)</th>
<th>AMP (μmol/g DW)</th>
<th>ADP/ATP</th>
<th>Pi (μmol/g DW)</th>
<th>CGP (μmol/g DW)</th>
<th>EC (μmol/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70-90%</td>
<td>27</td>
<td>18.0 ± 1.2</td>
<td>2.6 ± 0.5</td>
<td>96 ± 3</td>
<td>6.6 ± 2</td>
<td>0.5 ± 0.5</td>
<td>7.0 ± 3</td>
<td>0.021 ± 0.004</td>
</tr>
<tr>
<td>2</td>
<td>90-100%</td>
<td>27</td>
<td>21.1 ± 5.1</td>
<td>6.5 ± 0.4</td>
<td>6.1 ± 4</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>11.0 ± 5</td>
<td>0.022 ± 0.004</td>
</tr>
<tr>
<td>3</td>
<td>90-100%</td>
<td>27</td>
<td>21.5 ± 5.1</td>
<td>6.6 ± 0.4</td>
<td>6.1 ± 4</td>
<td>0.2 ± 0.2</td>
<td>0.1 ± 0.1</td>
<td>11.0 ± 5</td>
<td>0.022 ± 0.004</td>
</tr>
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</table>

Figure 1. Relation between the degree of coronary stenosis and the high energy phosphate pool (upper panel) and purine catabolites (lower panel) in the myocardium. Values are means ± SEM; * p < 0.05 versus group I; ** p < 0.01 versus group I; *** p < 0.001 versus group I. Σadenylates = sum of adenylates (adenosine tri-, di- and monophosphate); DW = dry weight. Upper panel: (●) Σadenylates; (○) creatine phosphate. Lower panel: (▲) adenosine + inosine; (●) hypoxanthine + xanthine.

Figure 2. Myocardial tissue content of adenine nucleotides, nucleosides and purine bases in group I patients with 90 to 100% stenosis without infarction (open bars) and in group III patients with evolving infarction (hatched bars). Values are means ± SEM. ** p < 0.01 versus 90 to 100% stenosis; *** p < 0.001. A + I = adenosine + inosine; ADP = adenosine diphosphate; AMP = adenosine monophosphate; ATP = adenosine triphosphate; DW = dry weight; H + X = hypoxanthine + xanthine; N₇N₅B = sum of nucleotides, nucleosides and bases.
only 0.1% of the sum of nucleotides, nucleosides and bases in mild stenosis, increased during acute coronary artery occlusion to 19%. The major fraction of the accumulated nucleosides was inosine: 2.65 ± 1.34 μmol/g or 91% of the nucleosides. The adenosine diphosphate (ADP)/ATP ratio, which increased slightly from 0.14 to 0.17 when the degree of stenosis progressed from mild to severe, increased further to 0.49 in acute coronary occlusion (Fig. 3). The energy charge (ATP + ½ ADP)/(ATP + ADP + adenosine monophosphate [AMP]) (10) decreased slightly from 0.921 to 0.904 when coronary stenosis progressed from mild to severe. After acute coronary occlusion the energy charge fell abruptly to 0.660 (Table 1 and Fig. 3).

Creatine phosphate decreased during acute occlusion to very low levels: myocardial tissue contents decreased to 17% of the value measured in mild stenosis (Fig. 1). After chronic infarction (group IV), purine content recovered to levels comparable with those in severe stenosis without infarction as did creatine phosphate, ADP/ATP ratio and energy charge (Table 1).

Histologic findings related to coronary artery stenosis (Table 2). At the light microscopic level, myocardial cell changes were quantified in subepicardial and subendocardial biopsy samples as described previously (9) and the percent of completely normal myocytes per sample was calculated. Myocardial cell abnormalities were characterized mainly by a reduction of the volume fraction of the myofibrils, especially in the perinuclear zones, and by nuclear tortuosity. The frequency of altered cells was significantly increased in patients of group II (90 to 100% stenosis without previous infarction). However, interpatient variation is very high in this group, which comprises patients with almost completely normal histologic findings as well as those with a high degree of myocardial cell abnormalities. Tissue samples from patients having ECG signs of previous anterior infarction (group IV) uniformly showed a low incidence of normal myocytes in the subepicardium and in the subendocardium: 32 and 17%, respectively.

Myocardial fibrosis was observed in only 11% of samples in group II. In group IV it was uniformly found in samples from patients with ECG signs of previous infarction.

At the ultrastructural level, the myocardium in samples from group I revealed a normal subcellular structure. In group II, ultrastructural changes consisted of myofibrillar atrophy, presence of many small mitochondria, glycogen accumulation and polymorphism of the nucleus (9,11) (Fig. 4). In some cases ultrastructure was completely normal. A normal subcellular structure was often associated with a history of unstable angina related to this area (Fig. 5).

Ultrastructural changes related to acute myocardial ischemia (1,12) were rare in all groups except group III (evolving myocardial infarction). In groups I, II and IV, only the lack of intramatricial granules in the mitochondria and some swelling of the mitochondrial matrix were noticed. In the group with evolving myocardial infarction (group III), ultrastructural degenerative changes were dramatic. In all subendocardial samples irreversible myocardial cell damage was found: rupture of the sarcolemma, mitochondria containing flocculent densities, intracellular edema and nuclear chromatin clumping (Fig. 6). In contrast, subcellular injury to the subepicardial cells was reversible: only mild to severe mitochondrial swelling without sarcosomal and nuclear changes was observed (Fig. 6).

Regional left ventricular wall motion (Table 2). When coronary stenosis progressed to 90%, segmental wall motion remained in the normal range. With stenosis of 90 to 100%,

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**Table 2. Myocardial Regional Wall Motion and Histology in 49 Patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>AWM* (%)</th>
<th>% Normal Myocytes*</th>
<th>Fibrosis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Epi</td>
<td>Endo</td>
<td>Total</td>
</tr>
<tr>
<td>Group I (n = 16)</td>
<td>48.9 ± 8.9</td>
<td>83 ± 14</td>
<td>79 ± 18</td>
</tr>
<tr>
<td>Group II (n = 27)</td>
<td>34.9 ± 9.9*</td>
<td>59 ± 33*</td>
<td>51 ± 30*</td>
</tr>
<tr>
<td>Group IV (n = 6)</td>
<td>24.8 ± 10.9*</td>
<td>32 ± 28*</td>
<td>17 ± 9*</td>
</tr>
<tr>
<td>F value</td>
<td>5.54*</td>
<td>6.61*</td>
<td>12.5*</td>
</tr>
</tbody>
</table>

Statistics as in Table 1. *Values are means ± SD. AWM = anterior wall motion; Epi = subepicardial layer; Endo = subendocardial layer.
Figure 4. Electron micrograph from a subendocardial biopsy sample of a hypokinetic anterior wall in a patient from group II. This section shows a severely altered myocyte. There is considerable reduction of the myofibrillar volume fraction in the center of the myocardial cell. The sarcolemma appears intact whereas the nucleus (n) is tortuous; most mitochondria are small in size and large areas of glycogen (gl) fill up the cytosol (original magnification x2,250, reduced by 25%).

Figure 5. Electron micrograph from a subendocardial sample of the anterior wall in a patient from group II with unstable angina. In this sample, myocardial structure is normal except for some clearing of the mitochondrial matrix (arrows) (original magnification x13,200, reduced by 25%).

however, wall motion declined to 72% of its initial value. With complete acute coronary artery occlusion, paradoxical movement of the segment occurred. This paradoxical movement was noted on the preoperative left ventriculogram in one case and was observed during surgery in the other four cases. In these four cases a left ventriculogram was not performed in the acute stage of evolving infarction. In group IV (patients with ECG evidence of previous infarction), segmental wall shortening attained only 50% of normal values.
Discussion

Myocardial purine metabolism in man. This study deals with the relation between coronary artery stenosis and myocardial purine metabolism, histology and regional function. In humans, this relation has not been previously established. Data on human myocardial tissue contents of nucleotides are scarce, and usually restricted to the measurement of adenosine triphosphate (ATP) (13). In experimental animals, on the other hand, purine metabolism and its catabolism during ischemia are relatively well known (1-5). Our clinical results show that high energy phosphate metabolism in humans is comparable with that found in experimental animals. Under normoxic conditions, the composition of the adenine nucleotide pool is similar in the human and the canine heart. We found that the adenosine diphosphate (ADP) and monophosphate (AMP) content in the human myocardium, expressed as a percent of the total adenylate pool, is 12 and 2%, respectively, when coronary artery stenosis does not exceed 90%. Comparable values are reported in experimental animals with normal coronary vessels (3-5, 14-17). The adenylate charge (10) in the human heart as reported in our study (0.921 ± 0.028, mean ± SD) is clearly in the same range as the values found in animal hearts (0.919 to 0.928) (3,4,14-16). Also the ratio between creatine phosphate and ATP (1.50 in this report) is comparable with values reported in animal studies (1.29 to 1.71) (3,4,14-16). Only values obtained with nuclear magnetic resonance techniques seem to produce a slightly higher creatine phosphate/ATP ratio (18).

Relation between coronary stenosis, purine metabolism, histology and regional function. Our results indicate that severe coronary stenosis is associated with a 20 to 30% reduction in myocardial nucleotide content. It may be assumed that in the presence of such severe stenosis, intermittent periods of myocardial ischemia lead to purine breakdown (15,17). Purine catabolism induces an accumulation of nucleosides and bases in the myocardium, but these substances are readily washed out as long as there is no complete occlusion of the vessel. Next, further catabolism to hypoxanthine represents an almost irreversible loss of the “backbones” of the adenylate pool. Such a washout of nucleosides and purine bases was shown previously by de Jong and Goldstein (19) and Kugler (20) when they obtained high levels of inosine and hypoxanthine in the coronary sinus blood during pacing and exercise-induced ischemic episodes. De novo synthesis of adenylylates is an extremely slow process (4), and it is probably not optimal in the presence of a severe coronary stenosis.

Severe left anterior descending artery stenosis was also associated with a 30% reduction in regional wall motion. This confirms the results of a previous study (7). Our observation that the reduction in the adenylate pool parallels the reduction in local contractility does not necessarily imply that the depression of local function is caused by this reduction in the adenylate pool. Indeed, the ADP/ATP ratio, the energy charge and the creatine phosphate levels are in the normal range and this is very suggestive for an intact mitochondrial function, that is, a normal energy production. We realize however that the ADP/ATP ratio, obtained by our methodology, is not an optimal estimate of mitochondrial function. The problem is that “cytosolic” ADP concentrations cannot be measured with any existing technology. Even with nuclear magnetic resonance techniques the ADP concentration can only be estimated by subtracting the ATP beta-phosphate peak from that of the gamma-phosphate peak and the error associated with this measurement is relatively high (21). Nevertheless, our values for creatine phosphate and energy charge are accurate and they are acceptable indexes of mitochondrial function.

A near normal energy production may well be associated with posts ischemic myocardial dysfunction, reduced adenylate pools and normal or elevated creatine phosphate levels (21,22). Experimental evidence exists that this entity, called myocardial stunning, is due to a deficiency of the creatine phosphate shuttle, at the level of the myofibrillar isoenzyme of creatine kinase (23). The same phenomena, reduced wall motion, reduced adenylate pool, normal mitochondrial function and normal creatine phosphate levels, were found in our group II patients; therefore, myocardial dysfunction may represent a “stunned myocardium” induced by chronic intermittent ischemia (6). However, recurrent episodes of ischemia not only may induce stunning and purine loss but also may activate proteolytic systems responsible for myofibrillar lysis. A certain degree of myofibrillary lysis was found in at least 50% of the myocytes in this subset of patients. We reported in previous studies (7,9,11) that this type of cell degeneration correlates best with reduction in left ventricular function. It was shown using immunohistochemical techniques (24) that fibers with myocytolysis are viable because they retain enzymes and other proteins (creatine kinase MB fraction, myoglobin, lactate dehydrogenase [LDH]-I, aspartate aminotransferase [AST]). Also using immunofluorescence staining Hayakawa et al. (25) demonstrated a similar labeling intensity for Ca2+ and Mg2+ adenosine triphosphatase and tropomyosin in myocytolytic cells as in normal muscle cells. Thus, myocytolysis may be a reversible form of myocardial alteration that, although it does not necessarily lead to cell death and eventual myocardial fibrosis (24), may well induce myocardial dysfunction.

Factors producing variations of findings in severe stenosis group. In the severe stenosis group there was a wide scatter in the biochemical, histologic and functional data. Many factors may contribute to this variation: 1) A small biopsy specimen is assumed to be representative of a relatively large area. Hearse et al. (3) showed that there is already a large variation in biochemical values when a large
number of simultaneously obtained samples are removed from a homogeneously perfused subepicardial zone during the same cardiac cycle. 2) Analytic errors may be related to the small size of the biopsy specimens. 3) Assessment of the degree of stenosis is not completely accurate because the length of the stenotic segment is not taken into account. 4) The determination of regional wall motion as an index of regional contraction may be inaccurate. Axis shortening as such may not completely reflect segment movement, and previous medication (beta-adrenergic blocking agents) may influence contraction. There is also a time interval between angiography and surgery so that correct extrapolation of wall motion data to the moment of tissue sampling remains questionable. 5) The absence of electrocardiographic evidence of anterior infarction does not exclude the presence of anterior wall infarction, a factor that may account for some of the differences between the group with the highest grade stenosis and those with lesser degrees of stenosis. On the other hand, some of the variation may be real because coronary stenosis is a dynamic process: progression of the disease may be fast or slow, coronary spasm may influence the degree of stenosis from one minute to another and the oxygen supply and demand balance of the heart is not stable. This group still comprises some patients with normal purine levels, normal histologic features and normal wall motion. Therefore, actual biochemical and functional measurements may represent no more than a “snap shot” of an evolutionary process.

Myocardial purine catabolism during evolving infarction. Purine catabolism during evolving myocardial infarction in humans has not been described before. Our results indicate that, in patients with a history of coronary artery disease, the rate of purine catabolism during more than 2 hours of acute coronary occlusion is not the same as that found experimentally in the dog heart. In patients, adenosine triphosphate decreased to 43% of its initial value (that is, 95% stenosis). In contrast, in dogs, adenosine triphosphate dropped to 6% of its normal value after only 40 minutes of acute coronary artery occlusion (1). This difference in tolerance to ischemia is most probably due to the presence of a better developed collateral circulation in patients with coronary artery disease as compared with healthy experimental animals. However, the pattern of purine catabolism in patients with acute coronary obstruction is similar to that in dogs with acute coronary occlusion. Adenosine monophosphate increases and the nucleosides and bases accumulate extensively (Fig. 2). The main component of the nucleosides during occlusion is inosine: this component represents 90% of the nucleoside pool, the remaining 10% being adenosine. The histologic findings in acute infarction in humans are also comparable with those in dogs (1). Mainly, the subendocardial part of the myocardium is irreversibly damaged. This implies that the reduction of ATP in the viable portion of the myocardium at risk will be less than the evaluation of a transmural and partly necrotic sample may suggest.

In the subclass of patients with healed anterior infarction, the same level of nucleotides was found as in patients with severe stenosis. However, the incidence of cell degeneration was higher and myocardial fibrosis was uniformly observed. This fibrous replacement of myocytes may explain the extremely low values of regional contractile function in this subclass despite relatively high levels of high energy phosphates.

Conclusions. Progressive coronary artery stenosis appears to result in a partial destruction of the adenylate pool, probably as a result of recurrent short periods of purine catabolism. Mitochondrial function, as estimated by the ADP/ATP ratio, energy charge and creatine phosphate/ATP ratio is not impaired so that the associated reduction in regional contractile function cannot be explained by a decreased production of high energy phosphates. The energetic base of this type of hypokinesia is probably an impaired energy utilization with reduced ATP splitting at the myofibrillar level as in the postischemic reperfused “stunned” myocardium (22,23). A reduced volume fraction of myofibrils, however, can be demonstrated histologically and may be the basis for this partial loss of function. Sudden complete obstruction of a coronary vessel, however, results in a massive degradation of high energy phosphates and an accumulation of mainly inosine in the ischemic myocardium. Mitochondrial function is severely impaired, as reflected by a sudden complete loss of contractile function. The rate of adenine nucleotide degradation is much slower than would be expected from animal studies. After 170 minutes of occlusion, ultrastructural evidence of cell necrosis occurs in at least the subendocardial part of the myocardium at risk.

We express our gratitude to Agnes Goethuys for preparing the manuscript.

References


