Sudden unexpected death in young adults

Discrepancies between initiation of acute plaque complications and the onset of acute coronary death

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Aims To study the time relationship between the onset of coronary thrombosis and sudden unexpected cardiac death in young adults.

Methods and Results Hearts of 11 young adults (≤35 years), who had died within 1 h after onset of symptoms and presented with a coronary thrombotic occlusion were studied retrospectively for the type of underlying plaque complication and the time of onset of thrombus formation. In all cases tissue blocks were taken from the occluded artery and sectioned for microscopic evaluation. Of 11 culprit lesions 10 were mainly fibrocellular; only one was lipid-rich. Inflammatory cells were found in all plaques, albeit in highly variable amounts. Plaque erosion had occurred in nine; deep ruptures in two. Analysis of the plaque-related occluding thrombus revealed fresh thrombosis in three (both ruptured plaques and one erosion); the other eight, however, showed occlusion with different histological stages of organization of thrombus.

Conclusions Despite strict inclusion criteria for sudden death in these young adults, the majority must have had plaque instability for some time, since thrombus formation had occurred at least days to weeks prior to the acute event.

Introduction

Significant coronary atherosclerotic disease in young adults (≤35 years) is a relatively rare event and in many instances is associated with one or more of the major risk factors for atherosclerosis[1,2]. At the same time, however, many of these young high risk patients witness a sudden unexpected death, the percentage of which is much higher than that seen in older patients with known coronary heart disease[1–4]. The high incidence of fatal outcome of coronary atherosclerosis in young adults could relate to the lack of protective mechanisms in the myocardium, e.g. poorly developed collateral circulation, or alternatively could be due to a particular plaque composition. Indeed, it has been reported that the histopathological features of atherosclerotic plaques in young adults differ from those in older age groups. Several investigators reported extensive smooth muscle hyperplasia as the main feature of plaques underlying acute coronary complications in young adults, whereas large lipid cores and thrombus formation were seen less often as compared to older patients with similar manifestations of coronary heart disease[4–6].

Recently, Farb et al.[7] identified plaque erosion as the most frequently encountered type of plaque complication in young patients with sudden coronary death; among those, moreover, erosion showed preference for women. In these patients the underlying plaque contained extensive areas of smooth muscle hyperplasia, with only scant lipid cores and little inflammation. Given the particular nature of the most common underlying plaque morphology, and the fact that plaque erosion is often considered part of a smoldering inflammation underlying the syndrome of unstable
angiota, the question arises as to the time relationship between the plaque complication resulting in occlusive thrombosis and sudden death. At present little attention has been given to this aspect. For this reason we have analysed retrospectively our material of sudden cardiac death in young adults, following the definition for sudden cardiac death, recently set by a task force on sudden cardiac death of the European Society of Cardiology[8], as well as the criteria for diagnosing coronary heart disease as the cause of sudden death with a high probability score, outlined by Davies et al.[9]. In this study the main emphasis is on the histopathological characteristics of the culprit plaques including the type of plaque complication and, in particular, the time of onset of thrombus formation.

Methods

Patient characteristics

The cardiovascular registry and heart/vessel bank was searched for patients of 35 years or less, who had died suddenly within 1 h after the onset of cardiac symptoms. Since this study focused on the time relationship between acute plaque complications and thrombus formation only those patients were included in whom a diagnosis of sudden coronary cardiac death was made on the identification of a thrombosed coronary artery plaque, either mural or totally occlusive, and in whom other causes of acute (cardiac) death were excluded at autopsy. Not included in this study were patients with sudden coronary death due to a high grade stenotic plaque, but without thrombus. On the basis of these criteria 11 patients were enrolled. A summary of the available relevant clinical data is shown in Table 1. Of these patients the formalin stored hearts and tissue blocks of coronary arteries including the thrombosed culprit plaque were evaluated. In nine of them a coronary angiogram was made at the time of autopsy; in six patients there was a LDH stained myocardial slice available to evaluate recent myocardial infarction.

Tissue processing and histopathological procedures

In all cases tissue blocks containing the culprit lesion within the thrombosed coronary artery segment were available for microscopic study. The paraffin embedded blocks were serially sectioned and at 100 μm intervals stained with haematoxylin and eosin. Adjacent sections of interest were mounted for immunohistochemistry. Immunostaining was performed with the following primary monoclonal antibodies: anti-CD68, reactive with all macrophages, including macrophage foam cells (clone PG-M1, 1/100 dilution, DAKO) and antismooth muscle α-actin, reactive with vascular smooth muscle cells (clone 1A4, 1/200 dilution, DAKO). Sections for CD68 staining were pre-treated with heat-induced antigen retrieval using citrate buffer pH 6·0[10]. A three-step streptavidin biotin complex method was used, and immune complexes were visualized with 3,3 diaminobenzidine tetrachloride. In a selection of cases a double immunostaining method with both primary antibodies (CD68 and α-actin) was applied.

Plaque composition of the culprit plaques

In eight plaques the composition was fibrolipid with a substantial fibrous cap (lipid core 25–75% of the plaque cross section), two plaques were fibrous (lipid core <25%), and one plaque was lipid rich with a thin cap and a core of >75%. Fibrocellular tissue with closely packed α-actin positive smooth muscle cells was the most prevalent tissue composition of the fibrous cap in all cases, with focal sclerotic areas in a minority of cases. Calcifications were present in five cases. Inflammatory cells (CD68+ macrophages) were present in 10 out of 11 lesions in highly variable amounts, adjacent to the atheromas (as CD68+ foam cells) and/ or diffusely interspersed within fibrocellular tissues.

Results

The clinical records of the patients revealed that the age range was between 24–35 years (mean age 30·9 ± 4 years). Seven were men and four were women. Relevant data are summarized in Table 1.

Two patients had deceased instantly while practicing sports; the other nine patients had died suddenly without an exercise related event. Seven patients had one or more major risk factors for atherosclerosis, including cigarette smoking (n=4), hypercholesterolaemia (n=3), defined as cholesterol levels of >6 mmol . l⁻¹, hypertension (>140/90 mmHg; n=2), diabetes mellitus (n=1) or a family history of ischaemic coronary heart disease (n=3). In four patients the existing clinical data were insufficient to establish or rule out the existence of risk factors.

Trivascular coronary atherosclerosis was present in five patients. Six patients had a high grade stenosing plaque of more than 75% in one or more epicardial arteries. In four patients fibrous scars representing healed infarctions, were noticed in the histological slides of left ventricular myocardium. The LDH staining of myocardial slices had shown regional or diffuse lack of staining in six cases according to the autopsy report, but they were not reliable for revision at the time of the present study (time dependent loss of staining). Microscopic signs of recent myocardial infarction, featured by regional hypereosinophilia, contraction band necrosis, and early infiltration by neutrophils, were seen in the hearts of three patients.
Table 1  Summary of relevant clinical data and findings at autopsy of 11 patients with sudden coronary death due to a thrombosed atherosclerotic plaque

<table>
<thead>
<tr>
<th>No</th>
<th>Sex</th>
<th>Age years</th>
<th>Risk factors*</th>
<th>Cause of death/ Clinical information</th>
<th>LDH† stain</th>
<th>Histology</th>
<th>Localization</th>
<th>Type of complication</th>
<th>Thrombus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M/F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Macro</td>
<td>Micro</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>32</td>
<td>DM, hypertension, smoking</td>
<td>Ventricular fibrillation at home</td>
<td>+</td>
<td>LAD</td>
<td>Erosion</td>
<td>Mural</td>
<td>Partially organized</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>34</td>
<td>Not known</td>
<td>Found dead in mentally ill care center</td>
<td>+</td>
<td>RCA</td>
<td>Rupture</td>
<td>Occlusive Fresh</td>
<td>Micro</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>31</td>
<td>Cocaine abuse, smoking</td>
<td>Angina 30' prior to death</td>
<td>—</td>
<td>LAD</td>
<td>Erosion</td>
<td>Occlusive Partially organized</td>
<td>Micro</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>35</td>
<td>HC</td>
<td>Found dead at home</td>
<td>+</td>
<td>LAD</td>
<td>Erosion</td>
<td>Occlusive Fresh</td>
<td>Micro</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>33</td>
<td>Family history HC</td>
<td>Angina (at rest) minutes prior to death at home</td>
<td>+</td>
<td>LAD</td>
<td>Erosion</td>
<td>Occlusive Partially organized</td>
<td>Micro</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>27</td>
<td>Smoking</td>
<td>Acute death during sports</td>
<td>+</td>
<td>RCA</td>
<td>Erosion</td>
<td>Occlusive Organized</td>
<td>Micro</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>24</td>
<td>Smoking</td>
<td>Acute death during exercise</td>
<td>N.D. Recent MI</td>
<td>LAD</td>
<td>Erosion</td>
<td>Occlusive Organized</td>
<td>Micro</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>24</td>
<td>Not known</td>
<td>Found dead at home</td>
<td>N.D.</td>
<td>N.R.</td>
<td>Erosion</td>
<td>Occlusive Partially organized</td>
<td>Micro</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>34</td>
<td>Not known</td>
<td>Found dead</td>
<td>N.D.</td>
<td>LAD</td>
<td>Erosion</td>
<td>Occlusive Partially organized</td>
<td>Micro</td>
</tr>
<tr>
<td>10</td>
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<td>35</td>
<td>Family history HC, hypertension</td>
<td>Angina shortly prior to death</td>
<td>+</td>
<td>LCx</td>
<td>Rupture</td>
<td>Occlusive Fresh</td>
<td>Micro</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>31</td>
<td>Not known</td>
<td>“Hyperventilation” 1 week prior to death. Found dead outside hospital</td>
<td>N.D. Fibrous scars; recent MI</td>
<td>RCA</td>
<td>Erosion</td>
<td>Mural</td>
<td>Partially organized</td>
</tr>
</tbody>
</table>

*Risk factors: DM=diabetes mellitus; hypertension (>140/90 mmHg); HC=hypercholesterolaemia (>6 mmol. l⁻¹); Family history=family history of ischaemic heart disease.
†LDH stain: regional or diffuse unstained areas using a lactate dehydrogenase stain of myocardial slices at autopsy; + = unstained areas present; — = unstained areas absent, N.D. = not done; N.R. = not properly recorded in autopsy report.
Haemosiderin-laden macrophages, as a marker of old plaque haemorrhage, were not conspicuous.

**Plaque complications**

Acute plaque complications underlying the thrombus were classified histologically as superficial erosions of the plaque in nine of 11 cases; only two cases showed rupture of the plaque extending into the lipid core of the lesion, associated with intraplaque haemorrhage.

Microscopic evaluation of the thrombus revealed that three of 11 cases presented a recent thrombus, composed of platelets, fibrin and red cells\(^1\). In one the thrombus was associated with an erosion, in the other two with plaque rupture. Both plaque ruptures had occurred in plaques with a fibrous cap containing large amounts of inflammatory cells; the eroded plaque, showed thrombus in continuity with a superficial infiltrate of foam cell macrophages (Fig. 1). The remaining eight plaques with superficial erosions and superimposed thrombi showed infiltrates of (foam cell) macrophages adjacent to the thrombus mass in 6 cases (Fig. 2). However, eight of the total of nine cases with plaque erosion showed thrombus with signs of organization\(^1\), characterized by smooth muscle cell ingrowth at the base of the thrombus as confirmed with anti-a actin immunostaining (Fig. 2). In four of eight cases, moreover, capillary vessel and deposits of connective tissue, as shown by the elastic van Gieson stain, amidst thrombus material with variable lytic changes, were identified indicating that thrombus organization had been going on for a considerable time (days to weeks) (Fig. 3).

**Discussion**

This is a retrospective study, using hearts available from a cardiac registry, which has focused deliberately on the time of onset of coronary thrombosis in relation to the type of coronary plaque complication, in young adults with sudden unexpected cardiac death. Hence, this is not an epidemiological study and the study design does not cater for providing insight into the prevalence of coronary thrombosis in this subset of patients.

In our series of 11 patients the diagnosis sudden cardiac death was based on the identification of a coronary thrombus overlying an atherosclerotic plaque, with exclusion of other causes of death at autopsy, which results in a high probability score (90%) for acute coronary death\(^9\). This point of departure, in combination with strict criteria for acute death (i.e. within 1 h after the onset of symptoms), resulted in two observations of major interest: (1) a high number of plaque erosions (nine of 11) as the immediate cause of coronary thrombosis and (2) a substantial amount of organized thrombi (eight of 11) overlying the culprit plaques, indicating initiation of thrombus formation several days up to more than a week before acute death.

In the recent past, major improvements have been made in the understanding of the onset of acute coronary syndromes. The immediate cause in most cases is a complication of an atherosclerotic plaque, which can be either a deep rupture in a plaque or a superficial erosion of the endothelial surface of an otherwise intact plaque. Several studies devoted to coronary plaque complications have shown that the ratio of rupture vs erosion varies from about 75/25% to 60/40 %\(^12\)-\(^16\). To this end it should be kept in mind that these studies have been performed in different laboratories and often with different patient populations, particularly with respect to age, sex and risk factors. However, in certain subpopulations of patients, these ratios differ substantially. For example, a higher incidence of erosions is noticed in diabetics\(^15\) and in younger patients, particularly young females\(^17\). The high number of erosions in our study (nine of 11) appears to fit with these observations. In young adults who die suddenly due to coronary heart disease there is a high prevalence of risk factors for atherosclerosis and its acute manifestations; in our small series seven of 11 had major risk factors recorded.

The degree of stenosis plays a role in the onset of acute syndromes, in particular in those instances in which erosions are involved\(^7\),\(^12\); in our series, six of 11 patients had a high degree of stenosis (75% or more). In addition, however, the tissue composition of the plaque is considered highly important. Plaque composition in young patients who die of acute coronary artery disease differs from the older age groups by showing prevalence for a thick and highly cellular cap in which smooth muscle cells dominate. This phenomenon has been documented previously\(^4\)-\(^6\) and also shows from the present observations. A ‘classical’ rupture prone plaque (attenuated fibrous cap and large atheroma) was seen in only one case and, indeed, this plaque had ruptured. In our experience, intra-plaque inflammation plays a crucial role in the onset of both types of plaque disruption, albeit to varying degrees. In fact, careful step sectioning of the eroded fibrolipid and fibrous plaques revealed CD68-positive lipid-laden macrophages in the fibrous cap adjacent to the thrombus in seven of nine instances. Indeed, limited amounts of inflammation, compared to those encountered in ruptured plaques, have been described in cases of erosive complications\(^3\) but complete absence of inflammation at the site of erosion appears the exception rather than the rule. In our previous series of erosions we found inflammation in erosive plaques in six of eight cases, albeit less extensive than in the classical lipid-rich plaque which had developed deep ruptures\(^14\). The potential causative role for intra-plaque inflammation in the onset of rupture/erosion and thrombus formation, relates to the tissue degrading effects of various secretory products of the inflammatory cells\(^18\). In addition, lipid-laden macrophages are known also to produce the thrombus initiator tissue factor which increases the thrombogenicity of the plaque\(^19\),\(^20\).
Figure 1. Coronary artery with a concentric and almost circumferentially eroded atherosclerotic lesion and recent thrombus. (A) Cross section shows superficial erosion and superimposed fresh occlusive thrombus. (B) High magnification of the boxed area in (A), containing the thrombus-plaque interface, which reveals the fresh nature of the thrombus and the inflammatory cells including many foam cell macrophages underneath the thrombus. (Haematoxylin-eosin stain.)
Figure 2  Coronary artery with an eccentric atherosclerotic lesion with erosion and thrombus with recent organization. (A) Cross section of the artery. The area indicated by arrows is shown in higher magnification (in B and C). (B) Detail of the thrombus and adjacent plaque surface, with lytic changes in the thrombus. A few spindle-shaped cells with elongated nuclei (arrows) can be seen inside the thrombus (A and B, Haematoxylin eosin stain). (C) Anti-CD68 (blue), anti-α actin (brown) double immunostain identifies ingrowth of smooth muscle cells at the base of the thrombus (arrows). In the thrombus and in the adjacent plaque many blue stained macrophages and neutrophils are present.
Figure 3. Cross-section through a coronary artery with an eccentric fibrolipid plaque and superficial erosion with advanced organizing thrombus. (A) Cross-section revealing also highgrade stenosis. The area indicated by arrows is shown in high magnification in B and C. (B) Detail of the thrombus/plaque interface. Strands of fibrocellular and microvessels (arrows) can be seen inside the thrombus (A and B, Haematoxylin eosin stain). (C) An anti-SMC actin stain (brown) of a serial section adjacent to (B) confirms the presence of smooth muscle cells.
Acute coronary death and thrombus organization

A second and thus far not reported but important finding was obtained from our studies on histological ageing of the thrombus overlying the culprit plaques. Despite the strict definition of sudden cardiac death in all patients we found histological evidence of thrombus organization in eight of 11 patients, whereas in only three was the thrombus completely fresh (less than 1 day old, as was apparent from a composition of layered platelets, fibrin and red cells only). Fresh thrombus occurred in two ruptured plaques and in one case of plaque erosion. Deep plaque rupture with exposition of the thrombogenic lipid core leads more readily to massive thrombosis and, hence, acute occlusion of the lumen, which could explain why no thrombus organization was found in these cases. The findings of smooth muscle cell ingrowth at the base of the thrombus in four patients and more longstanding organization, apparent from additional collagen deposition and ingrowth of microvessels in the thrombus mass, in another four patients with erosions, indicates that the thrombus in these individuals must have been initiated days or even weeks before the onset of death. In symptomatic patients, in particular those presenting with unstable angina, smooth muscle cell proliferation initiated by thrombus is well documented\([1,22]\). The observations in this study, however, imply that coronary thrombosis associated with plaque erosion, like plaque rupture, may occur while being clinically silent. We are unaware whether this particular aspect of plaque erosion had already been emphasized. Alternatively, negligence of symptoms in young individuals, in whom a slight or moderate degree of chest pain may not readily be interpreted as coronary heart disease, also could explain the lack of correlation between the onset of coronary erosive lesions and the eventual dramatic event of sudden cardiac death.

References