Virchows Arch [Pathol Anat] (1986) 409:535–542

Virchows Archiv A

An autofluorescence method for the diagnosis of early ischaemic myocardial lesions

A systematic study on 732 autopsies, including 182 cases of sudden death

J. von Overbeck, P. Saraga, and D. Gardiol
Institute of Pathology (CHUV), CH-1011 Lausanne, Switzerland

Summary. A systematic study of an autofluorescence method is described to improve the early histological diagnosis of myocardial ischaemia. Our results on 732 autopsy cases including 182 cases of sudden death show that the autofluorescence examination of haematoxylin and eosin stained sections of the myocardium is not only reliable in the identification of recent ischaemic lesions, but contributes to a better histological evaluation. In 24 cases undetected by white light examination it allowed recognition of ischaemic lesions.

Key words: Myocardial ischaemia – Histopathologic diagnosis – Autofluorescence

Introduction

Ischaemic lesions of the myocardium can be recognized under the microscope after a latency period, the length of which depends on the method used. The investigation reported here is a systematic study of an autofluorescence method described as improving the early histological diagnosis of myocardial ischaemia (Carle 1981).

Materials and method

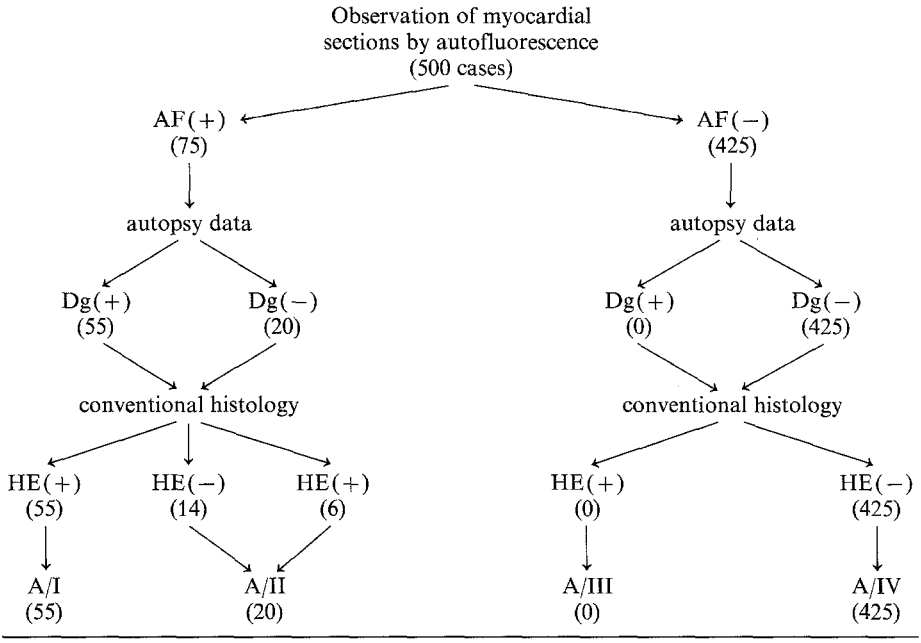
Myocardial samples from 732 autopsies of adults were used. In each case haematoxylin-eosin stained sections (3–6 blocks) were examined conventionally and by autofluorescence (Carle 1981) without reference to clinical or autopsy records. The results were correlated with the macroscopic data recorded in the autopsy reports, and with the clinical observations.

In order to evaluate the advantage and the limitations of the autofluorescence method 4 groups were formed.

Group A was made up of 500 consecutive autopsies of adults performed between January and December 1981. The steps followed in the study resulted in the definition of 4 sub-groups (Table 1).

Offprint requests to: P. Saraga at the above address

Table 1. Subgrouping of 500 consecutive autopsy cases with autofluorescence and routine histological examination of myocardium



AF(+)/(−): positive/negative to autofluorescence
Dg(+)/(−): ischaemic lesions described/not described in autopsy report
HE(+)/(−): ischaemic lesions observed/not observed in white light microscopy (second lecture)

Group B was made up of 182 autopsied cases of sudden or unexpected death, selected among the autopsies from 1970 to 1980 according to the following criteria: included, cases in which the symptoms (a) began 12 h or less before death, (b) changed markedly for the worse 12 h or less before death; excluded, cases in which (a) autopsy showed an extra-cardiac cause of death (Schwartz and Walsch 1971), (b) autopsy showed a cardiac noncoronary cause of death (Davies and Popple 1979, Schwartz and Gerrity 1975), (c) previous history documented severe cardiac arrhythmia.

Group C. A control group made up of 50 consecutive cases between 1972 and 1980 in which death occurred within 1 h after severe trauma and in which the heart at autopsy did not show any recent lesion.

Group D made up of 15 hearts. In order to determine the influence of autolysis on the results of histological examination by autofluorescence myocardial samples were taken at 12, 24, 36 and 48 h after death on (a) 10 hearts without acute ischaemic lesions (5 stored at room temperature; 5 at 4 degrees Celsius), (b) 5 hearts with recent infarction (kept at 4 degrees Celsius).

Histological criteria for ischaemic lesions. For the diagnosis of acute or subacute infarction the usual criteria were used (Fishbein et al. 1978; Lodge-Patch 1951; Mallory et al. 1939). A lesion showing an increased eosinophilia of myocardial fibres and a disseminated infiltration with polymorphonuclear neutrophils was termed early necrosis. Myocardial fibres showing

only slight increase in eosinophilia with or without margination of polymorphonuclears were not considered necrotic.

Autofluorescence criteria. Only lesions showing bright yellow fluorescence contrasting sharply with the olive green background were termed positive.

Symbols. Dg(+)/Dg(−)=ischaemic lesions described/not described in the autopsy report (white light microscopy); HE(+)/(−)=ischaemic lesions observed/not observed at a second lecture of slides (white light microscopy); AF(+)/(−)=positive/negative (autofluorescence examination); WLM=white light microscopy.

Results

Group A

This collective is made up of 295 men (average age at death 65 years) and 205 women (average age at death 69 years). Table 2 summarizes the correlations established between autopsy macroscopic data and the results obtained by WLM and AF.

Sub-group A/I: AF(+) Dg(+) HE(+), 55 cases

In this group both AF and WLM gave identical positive results. In 38 cases (69%) however fresh ischaemic lesions appeared much more sharply limited with AF than with WLM. In comparison with conventional microscopy, AF was clearly either positive or negative without transitional zones.

In 17 cases (31%) the AF(+) lesions were larger than the lesions visible in white light.

In 40 cases (72%) undulating myocardial fibres were observed. These fibres were AF(−) in 23 cases, AF(+) in 17 cases.

Table 2. 500 autopsy cases. Cardiac pathology data correlated with the 4 groups defined (Group A)

	A/I	A/II	A/III	A/IV	Total
Number of cases	55	20	0	425	500
Fresh coronary thrombosis (*n*)	20	0	0	6	26
ATS stenosis[a] (*n*)	18	2	0	35	55
Marked ATS[b] stenosis (*n*)	30	8	0	50	88
Old infarct (*n*)	21	6	0	49	76

[a] 50–75% stenosis of 1 to 3 vessels
[b] Greater than 75% stenosis of 1 to 3 vessels

A/I =AF(+) HE(+) Dg(+)
A/II =AF(+) HE(+/−) Dg(−)
A/III =AF(−) HE(+) Dg(+)
A/IV =AF(−) HE(−) Dg(−)

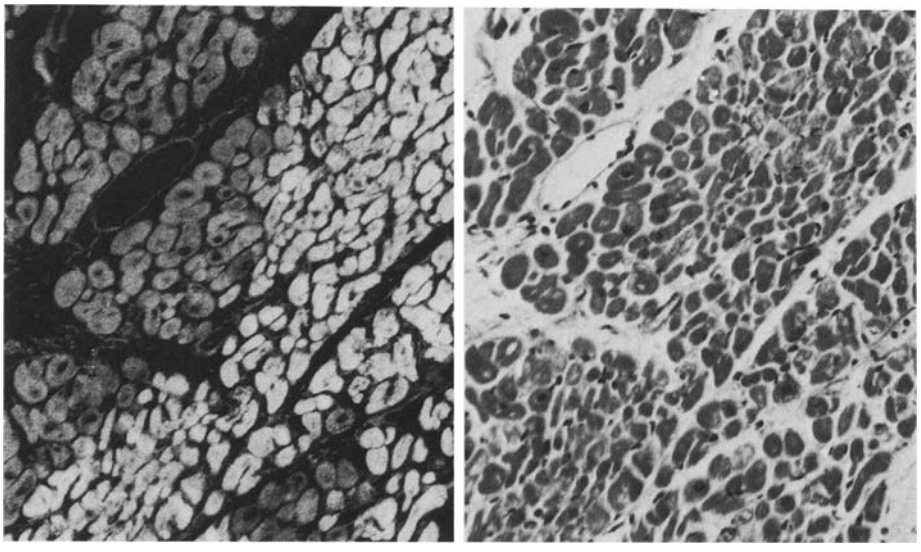


Fig. 1. Early myocardial infarct (H&E, ×140) (**a**) *short wave length blue light:* necrotic myocardial cells show distinct autofluorescence; the necrotic area is sharply limited. (**b**) *conventional white light:* no sharp limits are visible; presence of focal hypereosinophilia of the necrotic myocardial cells

Sub-group A/II: AF(+) Dg(−) HE(+/−), 20 cases

In this group AF(+) areas were observed but the autopsy reports mentioned no fresh ischaemic lesions of the myocardium. Histological reexamination disclosed signs of fresh necrosis in the AF(+) areas in 6 cases. In the 14 remaining cases no significant histological alterations were observed not even on the AF(+) areas. The clinical diagnosis in these 14 cases were: acute myocardial infarction (3×), atrial fibrillation (1×), severe cardiac failure (1×), ruptured aortic aneurysm (1×), death within a few hours after cardiac surgery (1×), shock syndrome (3×), traumatic subarachnoid haemorrhage (1×), brain disease (2×), bronchopneumonia (1×).

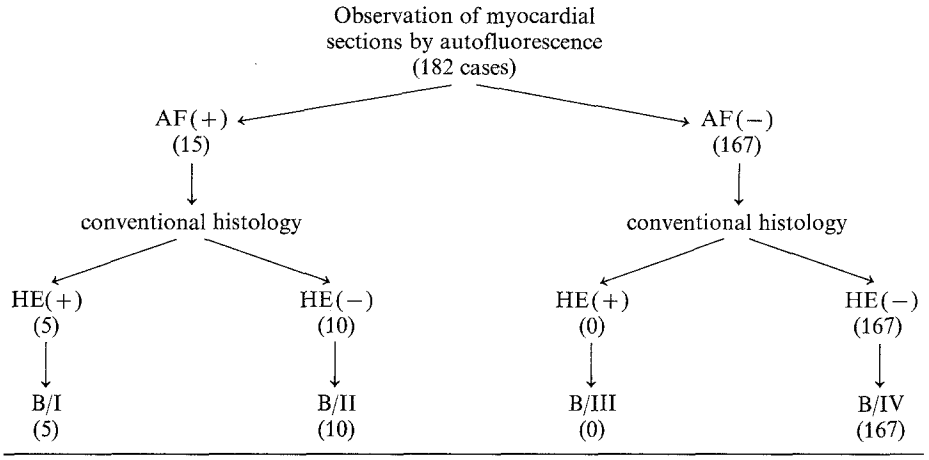
Sub-group A/III. AF(−) Dg(+) HE(+). No case corresponded to the defined criteria.

Sub-group A/IV: AF(−) Dg(−) HE(−). 425 cases. In this group no recent ischaemic lesion was observed in the myocardium with AF or WLM. In 5 cases the clinical diagnosis had been recent myocardial infarction, in 16 cases probable acute myocardial ischaemia was suspected.

Group B

Among the autopsies performed between 1970 and 1980 182 cases, 153 men (84%, average age at death 57 years), 29 women (16%, average age

Table 3. Subgrouping of 182 cases of sudden death with autofluorescence and routine histological examination of myocardium



AF(+)/(−): positive/negative to autofluorescence
HE(+)/(−): ischaemic lesions observed/not observed in white light microscopy (second lecture)

Table 4. 182 cases of sudden death. Cardiac pathology data correlated with the 4 groups defined (Group B)

	B/I	B/II	B/III	B/IV	Total
Number of cases	5	10	0	167	182
Fresh coronary thrombosis (*n*)	1	5	0	23	29
Marked ATS[a] stenosis (*n*)	5	9	0	73	87

[a] Greater than 75% stenosis of 1 to 3 vessels

B/I = AF(+) HE(+); B/II = AF(+) HE(−); B/III = AF(−) HE(+); B/IV = AF(−) HE(−)

at death 67 years), conformed to the criteria of selection. Table 3 summarizes the results obtained by WLM and AF examination of myocardium in group B. In Table 4 the correlations between autopsy cardiac macroscopic data and the results obtained by WLM and AF are summarized.

With reference to the time elapsed between the onset of the symptoms and death, 42% of the patients died within 1 h and 75% within 5 h. In 16% of the cases the exact time of death was not recorded. Fifteen cases were AF(+). In 5 of these (Group B/I) WLM revealed fresh myocardial necrosis. In 3 of the 5 cases the AF(+) areas were more extensive than the lesions observed in white light. In the remaining 10 AF(+) cases (Group B/II) there was no significant histological alteration of the myocardium in white light. In group B/II 5 of the 10 patients died within 5 h after onset of symptoms. In 5 cases there was fresh coronary thrombosis, in 5 cases severe atherosclerotic stenosis of the coronary arteries.

Group C

This control group comprised 39 men and 11 women (average age at death 44 years). In all cases examination of the myocardium gave negative results with both methods.

Autolysis study (Group D). Autolysis during the first 48 h after death does not seem to modify significantly the results obtained by autofluorescence. The average interval between death and autopsy was 14 h for collective A, 17 h for collective B and 27 h for collective C, with a maximum of 46 h.

Discussion

The study of collective A shows that all recent ischaemic lesions observed by conventional optical microscopy are positive with autofluorescence. Thus AF has excellent specificity for recent ischaemic damage in the myocardium, without any false negative. These results agree with those previously published (Al-Rufaie et al. 1983; Siegel and Fishbein 1982).

In 24 cases AF(−) Dg(−) HE(−) (14 cases in group A/II and 10 cases in group B/II), areas positive with AF were observed while WLM examination revealed no significant alteration. From a theoretical point of view these cases constitute "false positive" of the autofluorescence method. However we believe that these AF(+) areas very probably correspond to early ischaemic lesions not yet positive with conventional microscopy. In fact the clinical data in 23/24 cases are compatible with the presence of very recent ischaemic lesions of the myocardium (Baroldi 1975; Baroldi et al. 1979; Connor 1970; Greenhoot and Reichenbach 1969; Reichenbach and Moss 1975; Reichenbach et al. 1977; Scott and Briggs 1972): recent myocardial infarction in 8 cases, sudden cardiac arrhythmia in 3, unexpected death in 3, shock in 3, cardiac surgery in 1, ruptured aortic aneurysm in 1, severe heart failure in 1, intracranial disease in 3.

A clinical diagnosis of myocardial infarction was established in 5/425 cases from the groupe A IV (HE(−) AF(−)). Furthermore in 6/425 cases a fresh coronary thrombosis was found at post-mortem examination.

These cases could be interpreted as false negative results in early ischaemic lesions.

If autofluorescence positivity seems to appear before modifications are visible by WLM, the reverse has not been observed. Knowing that interpretable histological modifications are usually said to appear only 6 h after the onset of myocardial ischaemia (Fishbein et al. 1978; Lodge-Patch 1951; Mallory et al. 1939), we estimate that the latency period of the AF method lies between 4 and 6 h. In an experimental study Siegel and Fishbein (1982) have shown that early ischaemic lesions become apparent as hypereosinophilic areas positive with autofluorescence at 3 h after the onset of ischaemia. In 31% of the cases of group A/I HE(+)AF(+), the areas positive with AF are more extensive than the lesions observed with routine histological

examination. This discrepancy between the two methods is most noticeable on very early ischaemic lesions of the myocardium. AF reacts in an all-or-nothing manner. This property, common to other fluorescence methods, explains the sharp contrast observed between positive and negative areas (Hecht et al. 1961; Korb and Knorr 1962; Korb and Totovic 1963; Sahai and Knight 1976). In contrast the cytoplasmic eosinophilia of the myocardial cell increases progressively during ischaemia. This signs, in early ischaemia and in the absence of other histological alterations, may be difficult to perceive and to interpret. Thus, compared to routine histological examination, the autofluorescence method allows better visualization and delimitation of recent ischaemic lesions of the myocardium (Al-Rufaie et al. 1983). Consequently we cannot support Siegel and Fishbein's (1982) conclusions that the autofluorescence method is reliable but gives, in terms of microscopic identification, the same information as routine histological examination.

Our study of a large group (732 cases) including 182 cases of sudden death shows that the autofluorescence examination of haematoxylin and eosin stained sections of the myocardium is not only reliable in the identification of recent ischaemic lesions, but contributes to a better histological evaluation. In 24 cases it allowed recognition of undetected ischaemic lesions. In addition as it is more sensitive than routine histological examination it permits better screening at low magnification, particularly in cases of clinically suspected myocardial ischaemia. The method is also commendable for its simplicity.

References

Al-Rufaie HK, Florio RA, Olsen EGJ (1983) Comparison of the haematoxylin basic fuchsin picric acid method and the fluorescence of haematoxylin and eosin stained sections for the identification of early myocardial infarction. J Clin Pathol 36:646–649

Baroldi G (1975) Different types of myocardial necrosis in coronary heart disease: a pathophysiologic review of their functional significance. Am Heart J 89:742–752

Baroldi G, Falzi G, Mariani F (1979) Sudden coronary death. A postmortem study in 208 selected cases compared to 97 control subjects. Am Heart J 98:20–31

Carle BN (1981) Autofluorescence in the identification of myocardial infarcts. Hum Pathol 12:643–646

Connor RC (1970) Fuchsinophilic degeneration of myocardium in patients with intracranial lesions. Br Heart J 32:81–84

Davies MJ, Popple A (1979) Sudden unexpected cardiac death: a practical approach to the forensic problem. Histopathology 3:255–277

Fishbein MC, Maclean D, Maroko PR (1978) The histopathologic evolution of myocardial infarction. Chest 73:843–849

Greenhoot JH, Reichenbach DD (1969) Cardiac injury and subarachnoid hemorrhage. A clinical, pathological and physiological correlation. J Neurosurg 30:521–531

Hecht A, Korb G, David H (1961) Vergleichende histochemische, fluorescenzmikroskopische und elektronenoptische Untersuchungen zur Frühdiagnose des Herzinfarktes der Ratte. Virchows Archiv [Pathol Anat] 334:267–284

Korb G, Knorr G (1962) Vergleichende licht- und fluorescenzmikroskopische Untersuchungen frischer Herzmuskelschaden beim Menschen. Virchows Archiv [Pathol Anat] 335:159–164

Korb G, Totovic V (1963) Licht- und fluorescenzmikroskopische Befunde am Herzmuskel nach einer akuten, kurzfristigen Coronarinsuffizienz. Virchows Arch [Pathol Anat] 336:475–484

Lodge-Patch I (1951) The ageing of cardiac infarcts, and its influence on cardiac rupture. Br Heart J 13:37–42
Mallory GK, White PD, Salcedo-Salgar J (1939) The speed of healing of myocardial infarction. A study of the pathologic anatomy in seventy-two cases. Am Heart J 18:647–671
Reichenbach DD, Moss NS (1975) Myocardial cell necrosis and sudden death in humans. Circulation 51–52 (Suppl III): III-60-III-62
Reichenbach DD, Moss NS, Meyer E (1977) Pathology of the heart in sudden cardiac death. Am J Cardiol 39:865–872
Sahai VB, Knight B (1976) The post-mortem detection of early myocardial infarction by a simple fluorescent method. Med Sci Law 16:17–20
Schwartz CJ, Walsh WJ (1971) The pathologic basis of sudden death. Prog Cardiovasc Dis 13:465–481
Schwartz CJ, Gerrity RG (1975) Anatomical pathology of sudden unexpected cardiac death. Circulation 51–52 (Suppl III):III-18-III-26
Scott RF, Briggs TS (1972) Pathologic findings in pre-hospital deaths due to coronary atherosclerosis. Am J Cardiol 39:782–787
Siegel RJ, Fishbein MC (1982) Evaluation of fluorescence microscopy for the identification of necrotic myocardium. Human Pathol 13:1091–1094

Accepted February 10, 1986