# Altered fibrin clot properties in patients with chronic heart failure and sinus rhythm: a novel prothrombotic mechanism

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# Altered fibrin clot properties in patients with chronic heart failure and sinus rhythm: a novel prothrombotic mechanism

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## ABSTRACT

**Background** Thromboembolic complications occur more frequently in patients with chronic heart failure (CHF) than in the general population. Formation of a compact fibrin clot resistant to lysis has been shown in arterial and venous thrombosis.

**Objective** To investigate fibrin clot properties in patients with CHF.

**Method** Plasma clot permeability, compaction, turbidity and fibrinolysis were assessed in 36 consecutive patients with stable CHF (30M, 6F; aged 64±10 years, left ventricular ejection fraction (LVEF) 34.9±6.7%) and 36 controls matched for age, sex, cardiovascular risk factors and medication. Exclusion criteria were LVEF >40%, anticoagulant therapy, previous thromboembolic events, atrial fibrillation.

**Results** Clots obtained from plasma of patients with CHF had 23% lower clot permeability ($p<0.0001$), 13% less clot compaction ($p<0.001$), 15% faster fibrin polymerisation ($p<0.0001$) and tended to have prolonged fibrinolysis time ($p=0.1$) compared with controls. C-reactive protein and fibrinogen were associated inversely with clot permeability ($R^2=0.84$, $p<0.0001$ and $R^2=0.79$, $p<0.0001$, respectively) and positively with fibrinolysis time ($R^2=0.88$, $p<0.0001$ and $R^2=0.80$, $p<0.0001$, respectively) in patients with CHF. Plasma thrombin–antithrombin complex concentrations were inversely correlated with clot permeability ($R^2=0.88$, $p<0.0001$) and positively with fibrinolysis time ($R^2=0.91$, $p<0.0001$). Left atrium diameter, but not LVEF, correlated with fibrinolysis time ($R^2=0.61$, $p=0.027$).

**Conclusions** Patients with CHF with sinus rhythm are characterised by faster formation of compact plasma fibrin clots, which might predispose to thromboembolic complications.

## INTRODUCTION

Chronic heart failure (CHF) is a final common end point for a variety of the cardiovascular diseases, including coronary heart disease, arterial hypertension, valvular heart disease, primary and infectious cardiomyopathies. Signs and symptoms (shortness of breath and exercise intolerance) appear when the essential pump function is disturbed and are associated with poor outcome. Our understanding of the pathophysiology of CHF is evolving with emphasis on complex biochemical and neurohormonal interactions between endothelial, myocardial, resident and migrating cells and proinflammatory cytokines.[1]

Several factors contribute to the hypercoagulable state seen in CHF. These include decreased contractility, chamber dilatation resulting in decreased cardiac output and blood flow congestion being overlapped by haemostatic abnormalities. In 1979 Mehta *et al* showed that patients with congestive heart disease have more circulating platelet aggregates.[2] Elevated levels of platelet activation markers, such as β-thromboglobulin, platelet factor 4, P-selectin, platelet/endothelial cell adhesion molecule and osteonectin have been reported.[3–5] Increased plasma viscosity and concentrations of fibrinopeptide A, thrombin–antithrombin complexes (TAT), fibrinogen and D-dimer have also been observed in patients with CHF with sinus rhythm as well as atrial fibrillation.[3 4 6] Moreover, there is evidence for impaired endothelial function in CHF.[3]

It is well established that there is a higher risk of thromboembolic events in patients with CHF with or without concomitant atrial fibrillation.[7–10] Left ventricular ejection fraction (LVEF) appears to be independently associated with thromboembolic risk.[11]

The incidence of LV thrombus in patients with severe cardiomyopathy varies from 11% to 44% in the literature.[12] Several studies have shown that systemic and pulmonary emboli are more common in patients with ventricular thrombi. The annual risk of systemic embolisation in patients with dilated cardiomyopathy is 1.4–12.0%.[5 12 13] The MEDENOX study showed that 15% of patients with untreated heart failure develop venous thromboembolism within 2 weeks of a hospital admission, with the patients with more severe disease having a higher risk.[14] Moreover, Howell *et al* showed that CHF predicts venous thromboembolism in an outpatient population and the risk increases markedly as the LVEF decreases.[15]

Thrombi contain significant amounts of fibrin, the final product of the blood coagulation process, including thrombin-mediated fibrinogen conversion to fibrin and fibrin monomer cross-linking by activated factor (F)XIII.[16] A fibrin clot, which is characterised mainly by the thickness of fibrin fibres and the size of pores, has a major impact on fibrinolysis.[17]

It has been shown that formation of clots composed of compact thin fibrin fibre networks, which are resistant to fibrinolysis, predisposes to arterial thrombotic events. Reduced clot permeability and impaired fibrinolysis have been reported in patients with a history of myocardial

infarction,[18 19] cryptogenic ischaemic stroke[20] and deep vein thrombosis.[21]

The aim of this study was to evaluate fibrin clot properties in patients with CHF and normal sinus rhythm. We tested the hypothesis that in CHF fibrin clot properties are unfavourably altered and are similar to those seen in venous and/or arterial thromboembolism.[20 21]

## MATERIALS AND METHODS

### Study population

In this case–control study, 36 consecutive patients with diagnosed CHF were enrolled. Patients were eligible if they fulfilled the following criteria: diagnosis of heart failure,[22] LVEF <40% by echocardiography, haemodynamically stable condition with unaltered medication dosage within the 3 months before the study. An ischaemic aetiology of CHF was assumed in patients with a history of angiographically confirmed coronary artery disease (CAD), myocardial infarction and/or revascularisation. Subjects with history and tests (virus and parasite screening, antinuclear antibodies, rheumatoid factor, MRI, thyroid gland function) negative for secondary cause of heart failure were defined as idiopathic cardiomyopathy. The exclusion criteria were anticoagulation therapy, documented persistent or paroxysmal atrial fibrillation, acute coronary syndrome within 3 months preceding the study, severe decompensated heart failure, previous thrombotic event, bleeding tendency, renal insufficiency (serum creatinine >221 μmol/l) and known cancer.

Thirty-six volunteers matched for age, sex, cardiovascular risk factors and medication served as the control group. The exclusion criteria were the same as in the CHF group.

The Jagiellonian University Bioethical Committee (10 Jagiellońska Str, 31-010 Krakow, Poland) approved the study, and participants provided informed consent.

### Methods

#### Echocardiography

Standard transthoracic echocardiography was performed with a Acuson Sequoia C512 machine (Siemens Healthcare, Erlangen, Germany). The following parameters were evaluated: right ventricular diastolic diameter, left ventricular diastolic diameter, left ventricular systolic diameter, LVEF, interventricular septum diastolic diameter, left ventricular posterior wall diameter in diastole, left atrial systolic diameter and ascending aorta diameter.

#### Laboratory investigations

Fasting blood samples were drawn between 08:00 and 10:00 from an antecubital vein with minimal stasis. Routine blood tests, including lipid profile, blood cell count, glucose and serum creatinine, were carried out by automated laboratory techniques. Plasma samples (9:1 of 3.2% trisodium citrate) for the fibrin clot analysis were centrifuged (20 min, 2500 *g*) within 30 min of collection, immediately frozen and stored in aliquots at −80°C. Fibrinogen and high-sensitivity C-reactive protein (hsCRP) was measured by latex nephelometry (Dade Behring, Marburg, Germany). Commercially available immunoenzymatic assays were used to determine tissue-type plasminogen activator antigen (t-PA:Ag; Biopool, Ventura, California, USA), plasminogen activator inhibitor-1 antigen (PAI-1:Ag) (Biopool) and plasma TAT, a marker of thrombin formation (Dade Behring, Marburg, Germany). Coagulation factor VIII was measured by the one-stage clotting assay using factor-deficient plasma (Dade Behring, Liederbach, Germany).

All fibrin parameters described below were measured by an investigator blinded to the sample origin.

#### Fibrin clot permeability

Fibrin clot permeation properties were determined as previously described.[23 24] Briefly, 20 mmol/l calcium chloride and 1 U/ml human thrombin (Sigma, St Louis, MO, USA) were added to 120 μl of citrated plasma. After incubation in a wet chamber for 120 min, tubes containing the clots were connected to a reservoir of a buffer (10 mmol/l: 0.05 M Tris-HCl, 100 mmol/l: 0.15 M NaCl, pH 7.5) and its volume flowing through the gels was measured within 60 min. A permeation coefficient ($K_s$), which indicates the size of fibrin clot pores, was calculated from the equation: $K_s = Q \times L \times \eta / t \times A \times \Delta p$, where Q is a flow rate in time t, L is the length of a fibrin gel (13 mm), $\eta$ is the viscosity of the liquid (1/100 poise), A is a cross-sectional area (0.049 $cm^2$) and $\Delta p$ is a differential pressure (in dyne/$cm^2$). The interassay and intra-assay coefficients of variation were 8.6% and 6.9%, respectively.

#### Compaction

Citrated plasma was mixed (3:2) with 0.7 IU/ml thrombin, 0.1% Tween 80 and 20 mM calcium chloride in Tris-buffered saline, and then clots were formed in tubes prepared as in the permeation experiments.[23 24] After centrifugation at 6000 *g* for 60 s, the volume of the supernatant evacuated from the tubes was assessed by measuring the difference in weight of the tube. Compaction was expressed as this volume divided by the *initial* plasma volume used to form the fibrin clot. The interassay and intra-assay coefficients of variation were 7.7% and 6.5%, respectively.

#### Turbidity measurements

Plasma samples were diluted 1:1 with 50 mmol/l Tris-HCl, 150 mmol/l NaCl, pH 7.4 and by addition of 1 U/ml human thrombin (Sigma) and 15 mmol/l calcium chloride to plasma-initiated polymerisation. Absorbance was read at 405 nm for 15 min with a Perkin–Elmer Lambda 4B spectrophotometer (Molecular Devices , Menlo Park, California, USA). The lag phase of the turbidity curve, which reflects the time required for lateral aggregation, and maximum absorbance at a plateau reached by all subjects (ΔAbs), which reflects the number of protofibrils per fibre, were recorded.[23] The interassay and intra-assay coefficients of variation were, for the lag phase, 7.5% and 6.2%, respectively, and for ΔAbs, 7.4% and 6.4%, respectively.

#### Plasma clot lysis assays

Plasmin-mediated fibrinolysis in the presence of recombinant tissue plasminogen activator (rt-PA; Boerhinger Ingelheim, Germany) was evaluated as previously described.[23 24] Briefly, 100 μl of citrated plasma was diluted with 100 μl of a buffer (10 mmol/l: 0.05 M Tris-HCl, 100 mmol/l: 0.15 M NaCl, pH 7.4), containing 20 mmol/l calcium chloride, 1 U/ml human thrombin (Sigma) and 1 μmol/l rt-PA. The assembly kinetics was monitored by spectrophotometry at 405 nm in duplicate aliquots. Lysis time was defined as the time required for a 50% decrease in fibrin clot absorbance ($t_{50\%}$) and was chosen as a marker of the clot susceptibility to fibrinolysis. The interassay and intra-assay coefficients of variation were 8.3 and 6.9%, respectively.

### Statistical analysis

Data are expressed as mean ±SD or median (IQR) or as otherwise stated. The Kolmogorov–Smirnov test was used to determine normal distribution. Differences in variables between patients and controls were analysed by the $\chi^2$, Student t test or the Mann–Whitney U test, as appropriate. Logistic regression analysis was performed including demographic, laboratory and echocardiographic parameters. Pearson and Spearman correlation

coefficients were used to tests correlations between fibrin clot properties and echocardiographic parameters and other circulating markers. A p value <0.05 was considered statistically significant.

The study was powered to have an 80% chance of detecting a 10% difference in clot permeability ($K_s$)—the primary laboratory outcome measure—between the two groups using a p value of 0.05 based on previous papers.[21 23] In order to demonstrate such a difference or greater, 31 patients were required in each group.

## RESULTS

The CHF and control groups did not differ with respect to age, sex distribution, cardiovascular risk factors and most routine laboratory parameters (table 1). Causes of CHF were ischaemic heart disease (n=32, 89%) and idiopathic cardiomyopathy (n=4, 11%) (table 1). In the CHF group one patient was in New York Heart Association class I, 26 were in class II and nine in class III. More patients with CHF were treated with diuretics and eight of them used amiodarone, while all other drugs were administered in both groups with the same frequency (table 1). The patient and control groups differed in triglycerides and high-density lipoprotein-cholesterol, which were lower in the former group. Moreover, patients with CHF had significantly higher fibrinogen, creatinine and t-PA levels (table 1).

**Table 1** Characteristics of patients with chronic heart failure (CHF) and controls

| | CHF (n=36) | Controls (n=36) | p Value |
|---|---|---|---|
| Age (years) | 66.5 (46–80) | 64 (50–76) | 0.66 |
| Sex (M/F) | 30/6 | 30/6 | 0.99 |
| BMI (kg/m$^2$) | 27.48±3.89 | 26.92±3.36 | 0.53 |
| Smoking, n (%) | 5 (14) | 10 (28) | 0.23 |
| Hypertension, n (%) | 23 (64) | 30 (83) | 0.09 |
| Diabetes, n (%) | 7 (19) | 9 (25) | 0.57 |
| Previous MI or coronary artery disease, n (%) | 32 (89) | 30 (83) | 0.92 |
| Aspirin, n (%) | 34 (94) | 30 (83) | 0.13 |
| Statins, n (%) | 34 (94) | 30 (83) | 0.13 |
| ACEI, n (%) | 27 (75) | 30 (83) | 0.51 |
| ARB, n (%) | 13 (36) | 12 (33) | 0.74 |
| β Blockers, n (%) | 33 (92) | 30 (83) | 0.14 |
| Diuretics, n (%) | 22 (61) | 14 (39) | 0.03 |
| Digoxin, n (%) | 1 (3) | 0 | 0.30 |
| Amiodarone, n (%) | 8 (22) | 0 | 0.002 |
| Total cholesterol (mmol/l) | 4.48 (3.03–11.10) | 4.77 (3.19–6.57) | 0.17 |
| LDL-C (mmol/l) | 2.73 (1.59–8.76) | 3.0 (0.89–4.45) | 0.07 |
| HDL-C (mmol/l) | 1.15±0.24 | 1.32±0.30 | 0.01 |
| TG (mmol/l) | 1.48 (0.77–2.91) | 1.84 (0.51–2.78) | 0.03 |
| Glucose (mmol/l) | 5.30 (3.10–10.50) | 5.02 (4.38–6.10) | 0.08 |
| Creatinine (μmol/l) | 95.0 (60–182) | 79.25 (60–119) | 0.02 |
| CRP (mg/l) | 2.48 (0.58–12.71) | 1.79 (0.66–7.20) | 0.12 |
| Fibrinogen (g/l) | 4.24±1.36 | 3.21±0.92 | <0.0001 |
| Platelets (x10$^9$/l) | 208.69±45.16 | 210.14±29.63 | 0.87 |
| t-PA (ng/ml) | 10.91±2.73 | 9.65±2.13 | 0.03 |
| PAI-1 (ng/ml) | 15.7 (5.6–40.2) | 16.7 (8.5–21.4) | 0.95 |
| TAT (μg/l) | 4.22±1.64 | 3.76±1.39 | 0.20 |
| FVIII (%) | 121.28±25.91 | 118.16±27.65 | 0.62 |

Data are given as mean±SD (Student t test), median (IQR) (Mann–Whitney test) or number (percentage) ($\chi^2$ test) as appropriate.
ACEI, ACE inhibitors; ARB, angiotensin AT1 receptor blockers; BMI, body mass index; CRP, C-reactive protein; F, coagulation factor; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MI, myocardial infarction; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin-antithrombin complexes; TG, triglycerides; t-PA, tissue-type plasminogen activator.

As expected, the patients with CHF were characterised by lower LVEF and increased diameters of the heart chambers, interventricular septum and aorta (table 2).

Analysis of plasma fibrin clots showed that the patients with CHF had 23% lower clot permeability, 13% lower clot compaction, 15% faster fibrin polymerisation and non-significant tendency to prolonged fibrinolysis time as compared with controls (table 3). There was no significant difference in fibrin fibre thickness between the groups reflected by maximum absorbency in turbidimetry (table 3).

Age had no association with any of fibrin clot parameters in patients with CHF, whereas in controls age was correlated positively with fibrinolysis time ($R^2$=0.80, p<0.0001) and inversely with clot permeability ($R^2$=0.84, p<0.0001). There were no significant differences of fibrin parameters between male and female patients in either groups.

Smoking was the only cardiovascular risk factor in the CHF group which was associated with fibrin clot properties. Current smokers tended to have decreased clot permeability (5.74±1.34 vs 6.76±0.98 $\times 10^{-9}$ cm$^2$, p=0.052 for smokers and non-smokers, respectively) and prolonged fibrinolysis time (10.74±1.93 vs 9.59±1.16 min, p=0.078); neither differences achieved statistical significance. Interestingly, in the control group hypertension was associated with increased fibrin fibre thickness (ΔAbs, 0.99±0.1 vs 0.77±0.04, p<0.0001) and decreased clot compaction (47.4±5.1 vs 69.8±6.1%, p<0.0001). No such differences were seen in the patients with CHF (data not shown).

C-reactive protein and fibrinogen were associated inversely with clot permeability ($R^2$=0.84, p<0.0001 and $R^2$=0.79, p<0.0001, respectively) and positively with fibrinolysis time ($R^2$=0.88, p<0.0001 and $R^2$=0.80, p<0.0001, respectively) in patients with CHF. Plasma TAT concentrations were inversely correlated with clot permeability ($R^2$=0.88, p<0.0001 for patients with CHF and $R^2$=0.91, p<0.0001, for controls) and positively with fibrinolysis time ($R^2$=0.91, p<0.0001 and $R^2$=0.86, p<0.0001, respectively). The remaining laboratory variables showed no associations with any fibrin variables (data not shown).

Analysis of echocardiographic parameters showed that there was a positive relationship between left atrium diameter and fibrinolysis time ($R^2$=0.61, p=0.027). No fibrin clot properties showed associations with LVEF or other echocardiographic variables (data not shown).

## DISCUSSION

This study shows that CHF with sinus rhythm is associated with formation of less permeable and compactable fibrin clots as

**Table 2** Echocardiographic parameters in patients with chronic heart failure (CHF) and controls

| Parameters | CHF (n=36) | Controls (n=36) | p Value |
|---|---|---|---|
| LVEF (%) | 34.9±6.7 | 63.0±4.4 | <0.0001 |
| RVDD (mm) | 25.7±3.8 | 21.0±2.8 | <0.0001 |
| LVDD (mm) | 64.2±9.0 | 51.4±2.8 | <0.0001 |
| LVSD (mm) | 52.9 (20–71) | 36.5 (27–49) | <0.0001 |
| IVS (mm) | 9.0 (4–21) | 10 (7–14) | 0.03 |
| PW (mm) | 9.5±2.4 | 9.3±1.0 | 0.63 |
| Ao (mm) | 32.4±3.6 | 30.2±3.6 | 0.01 |
| LA (mm) | 42.0±5.4 | 37.1±3.5 | <0.0001 |

Data are given as mean±SD (Student t test), median (IQR) (Mann–Whitney test) as appropriate.
Ao, ascending aorta; IVS, interventricular septum; LA, left atrium; LVDD, left ventricular diastolic diameter; LVEF, left ventricular ejection fraction; LVSD, left ventricular systolic diameter; PW, posterior wall; RVDD, right ventricular diastolic diameter.

**Table 3** Fibrin clot properties in patients with chronic heart failure (CHF) and controls

| | CHF (n=36) | Controls (n=36) | p Value |
|---|---|---|---|
| $K_s$ ($10^{-9}$ $cm^2$) | 6.8 (4.9–8.6) | 8.85 (5.7–11.1) | <0.0001 |
| Compaction (%) | 44.72±4.68 | 51.11±9.97 | 0.001 |
| $t_{50\%}$ (min) | 9.77±1.31 | 9.08±2.11 | 0.1 |
| ΔAb max (405 nm) | 0.92±0.13 | 0.96±0.12 | 0.23 |
| Lag phase (s) | 37.97±3.49 | 44.53±4.06 | <0.0001 |

Data are given as mean±SD (Student t test), median (IQR) (Mann–Whitney U test) as appropriate.
ΔAb max (405 nm), maximum absorbance; $K_s$, denotes permeability coefficient; $t_{50\%}$, fibrinolysis time.

compared with well-matched controls. This association is due to the fact that smaller pores in fibrin network result in a stiffer fibrin configuration leading to less compactable clots.[21 25] Plasma obtained from the patient group formed fibrin clots much faster, which is shown by the shorter lag phase of the fibrin formation curve, and these clots tended to be lysed at a slower rate than in controls, although this difference did not reach statistical significance. Similar levels of PAI-1, which is major inhibitor of fibrinolysis, might in part explain this observation. Importantly, CAD was the main cause of CHF in this study. Since it has been previously reported that CAD is associated with lower clot permeability, faster fibrin polymerisation and prolonged fibrinolysis time,[23] we selected a control group that comprised a similar percentage of people with this disease as in the patient group. To our knowledge, there have been no published reports on the impact of haemodynamic and humoral changes in CHF on fibrin clot formation and degradation. Based on the data demonstrating that altered fibrin clot characteristics, which are similar to those reported in this study, are detectable in patients with ischaemic stroke [20] as well as deep vein thrombosis,[21] the current findings increase our knowledge on the prothrombotic state in CHF and possibly on the background of thromboembolic events in patients with CHF. To corroborate the hypothesis generated by our observations, a large study with long-term follow-up is needed.

We have identified several parameters associated with unfavourable plasma fibrin clot features in patients with CHF with sinus rhythm. In this population, clot permeability and fibrin clot lysis time were associated with the degree of inflammation as shown by CRP and fibrinogen levels. Inflammation is the prominent underlying factor of an unfavourable remodelling process taking place in the myocardium in CHF. Fibrinogen is recognised as the most important modulator of fibrin clot structure.[25] We confirmed that higher fibrinogen levels are related to reduced clot permeability and a trend towards impaired fibrinolysis also in patients with CHF. Although CRP levels did not vary significantly between the two groups, there were associations between CRP and the two fibrin parameters. Strong associations were detected between $K_s$ and $t_{50\%}$ and thrombin generation measured in circulating venous blood as plasma TAT concentrations, which is in line with our previous observations made in cardiovascular patients.[24] Compelling evidence indicates that the greater the thrombin formation, the more dense are the fibrin clots, which results in poor lysability.[25] Our study provides additional evidence for the adverse impact of current smoking on fibrin clot functions, which has been reported for asymptomatic subjects recently.[26]

Most echocardiographic parameters have no associations with fibrin clot properties in patients with CHF, except for left atrium diameter and fibrinolysis time. It is well established that the left atrium diameter predicts the onset of atrial fibrillation[27] and thus is an important risk factor of stroke. Our findings confirm that even in patients with sinus rhythm the left atrium size is associated with prothrombotic alterations, which may contribute to the risk of cerebral thromboembolic events.

It should be emphasised that adverse clot properties were apparent despite administration of aspirin and statins in a vast majority of both groups. It was previously documented that aspirin and statins can increase clot permeability and facilitate fibrinolysis.[24 25 28 29] However, in CHF these effects seem to be diminished owing to processes such as platelet activation, free radical formation and inflammation.

This study has several limitations. First, the number of the patients studied was small. However, both groups of equal size were well matched for potential confounders such as demographic and clinical variables, in particular, documented CAD. However, some subgroup analyses should be interpreted with caution. Second, our analysis was based on a determination of each variable at a single time point. It is likely that with time and changing humoral factors and drugs, clot features will undergo changes. Third, fibrin clot structure was not investigated by scanning electron microscopy, which requires clot dehydration. However, functional plasma-based assays appear to provide more valuable insights into the role of fibrin clots in human pathophysiology. Fourth, idiopathic cardiomyopathy represented a minority in this study and we cannot extrapolate the results of fibrin tests on this population of patients with CHF. Finally, statistical associations reported here do not necessarily mean a cause–effect relationship. Further studies with long-term follow-up performed in patients with CHF and an appropriate number of thrombotic events are needed to validate the observations.

In conclusion, our findings demonstrate that altered fibrin clot structure/function is seen in patients with CHF with normal sinus rhythm. The study demonstrates a novel potential prothrombotic mechanism, which might contribute to pathogenesis of the thrombotic arterial and venous complications of CHF.

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**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** This study was conducted with the approval of the Jagiellonian University Ethical Committee 10 Jagiellońska Str, 31-010 Cracow, Poland.

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