Inflammasome activation in end-stage heart failure-associated atrial fibrillation

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Abstract

Aims Inflammatory pathways are increasingly recognized as an important factor in the pathophysiology of both heart failure (HF) and atrial fibrillation (AF). However, there is no data about inflammation-related histological and molecular alterations in HF-associated AF. The objective of our study was to investigate inflammatory pathways and fibrosis in end-stage HF-associated AF.

Methods and results Left atrial samples of 24 male patients with end stage ischemic HF undergoing heart transplantation were analysed. Twelve patients suffered from sustained AF while the others had no documented AF. The expression of inflammasome sensors and their downstream signalling were investigated by Western blot. No differences were observed in the expression of inflammasome sensors between the two groups, while cleaved caspase-1 increased tendentially in the AF group (P = 0.051). Cleaved caspase-1 also showed significant correlation with the expression of interleukin-1β and its cleaved form in the total population and in the AF group (P < 0.05). The presence of myocardial and epicardial macrophages were assessed by ionized calcium-binding adaptor molecule 1 (Iba1) immunostaining. Number of macrophages showed a tendency towards elevation in the left atrial myocardium and epicardium of AF compared with SR group. The amount of total and interstitial fibrosis was determined on Masson’s trichrome-stained sections. Histological assessment revealed no difference between AF and SR groups in the amount of either total or interstitial fibrosis.

Conclusions This is the first study on inflammation-related differences between HF with SR or AF showing elevated inflammasome activity and enhanced macrophage infiltration in left atrial samples of patients with AF.

Keywords Heart failure; Atrial fibrillation; Inflammasome; Macrophages; Fibrosis

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Background

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. Heart failure (HF) is a known risk factor for AF; however, numerous HF patients never develop AF indicating presumable pathological differences between the types of HF associated with AF or sinus rhythm (SR). There is a growing number of evidence that inflammatory mechanisms contribute to the pathogenesis of AF.1,2 Although inflammasome activity is observed among patients with HF generally,3 there is lack of publications investigating whether the presence of AF is associated with enhanced inflammation in atrial tissues of HF patients.

Aims

We aimed to assess whether there is a relationship between inflammation and HF-associated AF in failing human
hearts. The focus of our research was on canonical inflamma-
some activation.

Methods

De-identified human left atrial samples from explanted hearts of 24 patients with end-stage ischemic HF undergoing heart transplantation were obtained from the Transplantation Biobank of the Heart and Vascular Center at Semmelweis University, Budapest, Hungary. The project complies with the Declaration of Helsinki and it was approved by the institutional and national ethics committee (ethical permission numbers: ETT TUKEB 7891/2012/EKU (119/PI/12.) and ETT TUKEB IV/10161–1/2020). All patients provided written in- formed consent. Half of the patients had no documented AF (SR, n = 12) and the other half suffered from persistent AF (AF, n = 12). All individuals were male between 43–64 years of age (SR: median 56.5 [IQR 48–60] years, AF: median 57.5 [IQR 54.5–60.5] years) with body mass index of 18.2–33.2 kg/m² (SR: median 28.9 [IQR 25.0–30.2] kg/m², AF: median 25.8 [IQR 23.5–28.8] kg/m²), ejection fraction of 10–36% (SR: median 20 [IQR 18–25]%, AF: median 24 [IQR: 22–26]%) and left atrial length of 47-82 mm (SR: median 58 [IQR 52–65]mm, AF: median 63 [IQR 59–66]mm). None of them suffered from diabetes mellitus (Table 1).

Inflammasome activation was assessed by Western blot from tissue lysates prepared from left atrial samples as previously described. The expression of inflammasome sensors (NLR family, pyrin domain containing 1 and 3 [NALP1, NLRP3], absent in melanoma 2 [AIM2], NLR family CARD domain-containing protein 4 [NLRC4]) and their downstream signalling (apoptosis-associated speck-like protein containing a CARD [ASC], caspase-1, interleukin-1β) were analysed (Supplementary Figure). Five samples (two from SR and three from AF) were excluded due to low-quality homogenates (Figure 1A). Image analysis was performed using Image Lab™6.0 software (Bio-Rad, Hercules, CA, USA).

To assess the presence of macrophages in the epicardial and myocardial areas of left atrial samples (Figure 2A) from SR (myocardium: n = 6, epicardium: n = 4) and AF (myocardium: n = 7, epicardium: n = 6) groups, immunohistochemistry was performed to stain ionized calcium-binding adaptor

| Table 1 Patient characteristics | Patients with sinus rhythm (n = 12) | Patients with atrial fibrillation (n = 12) |
|-------------------------------|-----------------------------------|------------------------------------------|
| Age (years)                   | 56.5 (48.0–60.0)                  | 57.5 (54.5–60.5)                         |
| Sex (M/F)                     | 12 (100)/0 (0)                    | 12 (100)/0 (0)                           |
| BMI (kg/m²)                   | 28.9 (25.0–30.2)                  | 25.8 (23.5–28.8)                         |
| Aetiology of heart failure    |                                   |                                          |
| Ischemic                      | 12 (100)                          | 12 (100)                                 |
| Non-ischemic                  | 0 (0)                             | 0 (0)                                    |
| NYHA stage                    |                                   |                                          |
| II                            | 0 (0)                             | 1 (9)                                    |
| III                           | 7 (58)                            | 7 (64)                                   |
| IV                            | 5 (42)                            | 3 (27)                                   |
| Echocardiography parameters   |                                   |                                          |
| EF (%)                        | 20 (18–25)                        | 24 (22–26)                               |
| LA length                     | 58 (52–65)                        | 63 (59–66)                               |
| Artificial heart valve        | 0 (0)                             | 1 (8)                                    |
| CRT                           | 3 (25)                            | 5 (42)                                   |
| Medication                    |                                   |                                          |
| Parenteral loop diuretics     | 3 (25)                            | 4 (33)                                   |
| Parenteral inotropic support  | 3 (25)                            | 4 (33)                                   |
| Parenteral vasopressor agent  | 0 (0)                             | 1 (8)                                    |
| Mechanical circulatory support| 1 (8)                             | 1 (8)                                    |
| Mechanical ventilation        | 0 (0)                             | 1 (8)                                    |
| Ventricular tachycardia       | 1 (8)                             | 4 (33)                                   |
| Diabetes mellitus             | 0 (0)                             | 0 (0)                                    |
| Infection                     |                                   |                                          |
| Presence of infection         | 4 (33)                            | 4 (33)                                   |
| Antibiotic treatment due to infection | 3 (25)                        | 4 (33)                                   |
| White blood cell count (G/L)  | 7.6 (6.7–9.5)                     | 8.9 (6.6–9.4)                            |
| CRP (mg/L)                    | 4.7 (1.6–9.7)                     | 3.0 (2.4–4.4)                            |
| PCT (µg/L)                    | 0.03 (0.02–0.05)                  | 0.10 (0.05–0.13)                         |

The most relevant baseline characteristics of the patients whom left atrial tissues were examined are listed. All patients were male with ischemic cardiomyopathy. In case of categorical variables, data are presented as number (%), while in case of continuous variables, median values (interquartile range) are shown.

BMI, body mass index; CRP, C-reactive protein; CRT, cardiac resynchronisation therapy; EF, ejection fraction; F, female; LA, left atrial; M, male; NYHA, New-York Heart Association; PCT, procalcitonin.

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molecule 1 (Iba1, marker of monocyte–macrophage lineage). An outlier was identified by ROUT test (Q = 1%) in the epicardial subgroup of SR and was excluded from data analysis.

The amount of total and interstitial fibrosis was determined on Masson’s trichrome-stained sections (SR: n = 6, AF: n = 7). Percentage of total fibrosis was defined as the amount of fibrotic regions compared with the whole area of the section, while the amount of interstitial fibrosis was correlated to a modified area which did not include the epicardium, subepicardial fibrotic area and fibrosis surrounding vascular structures (Figure 3A). An outlier at the interstitial fibrosis subgroup of SR was excluded from statistical analysis.

Stained sections were visualized and images were acquired using Leica DM3000 bright field microscope (Leica Microsystems, Wetzlar, Germany). For fibrosis and macrophage quantification, ImageJ (Image Processing and Analysis in Java) 1.51 k program was applied. Continuous data were presented as mean ± standard error of the mean or median with interquartile range. Categorical data were presented as numbers (percentages). Comparisons of two groups were performed using unpaired Student’s t-test. All analyses were
Conducted using GraphPad Prism 8. (GraphPad Software Inc). We considered a $P$-value of $<0.05$ to be statistically significant.

**Results**

No significant differences were observed in the expression of any types of inflammasome sensors between AF and SR groups. There was a strong tendency that cleaved caspase-1 was increased in the AF group versus the SR group ($P = 0.051$) (Figure 1B). Furthermore, it showed significant correlation with the expression of interleukin-1β and its cleaved form ($P < 0.05$). This correlation was observed in the total population and in AF group alone but not in SR group. In contrast, cleaved caspase-1 level showed no correlation with the expression of inflammasome sensors, suggesting overall a primary effect on inflammasome activity rather than on inflammasome priming (Figure 1C).
We found enhanced macrophage presence in the left atrial myocardium of AF compared with SR group. Likewise, macrophages were present in a higher number in the left atrial epicardium of AF than of SR. Neither differences were however significant due to high variability (Figure 2B,C).

Histological assessment of left atrial samples revealed no relevant difference between AF and SR groups in the amount of total fibrosis and interstitial fibrosis (Figure 3B,C).

Discussion and conclusions

Inflammasomes are well-characterized signalling complexes that regulate immune responses. Their activation involves priming (transcription of inflammasome components and effectors) and triggering (enhancing the assembly of the inflammasome complex). Elevated protein levels of active caspase-1 and NLRP3 as well as enhanced macrophage infiltration in atrial tissue were reported in chronic and postoperative AF, suggesting that NLRP3 inflammasome activation might contribute to these phenomena.\(^1,2\) Additionally, some previous studies verified elevated inflammatory markers (C-reactive protein, interleukin-6, tumour necrosis factor-alpha) in venous blood samples of HF patients suffering from AF compared with HF patients with SR,\(^4,6\) while another group showed that patients with typical (paroxysmal/persistent/permanent) AF had significantly elevated serum concentrations of interleukin-10 and tumour necrosis factor-alpha compared with patients with AF only. This result was explained by the relatively high (20%) presence of HF in typical AF group compared with the absence of any structural heart diseases in the case of lone AF.\(^7\) Elevated serum levels of high-sensitivity C-reactive protein and interleukin-6 were demonstrated to strongly associate with the risk of HF-related hospitalization among patients with AF.\(^8\) The current research was the first that analysed inflammation-related differences between HF patients with SR and AF in cardiac tissue samples. In our study, protein levels of inflammasome sensors were unaltered between AF and SR groups, indicating unaffected priming signal. However, tendency towards increase in the expression of cleaved caspase-1 and its significant correlation with the expression of (cleaved) interleukin-1β in AF samples suggest enhanced triggering for inflammasome activation in end-stage HF-associated AF.

The main limitation of this study is the lack of enough evidence that inflammasome activity contributes directly to maintaining AF in patients with end-stage HF. Further studies...
are needed to define any causalities, correlations and consequences, to determine possible prognostic factors and to evaluate whether inflammasome activation has a diagnostic role in end-stage HF-associated AF.

In conclusion, this is the first study on inflammation-related differences in failing human hearts with SR and AF that analysed cardiac tissues. It verified that inflammasome activity may associate with AF in patients with end-stage HF. Enhanced macrophage infiltration also indicates higher levels of inflammation in AF samples, while HF-associated AF may be independent of cardiac fibrosis.

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Conflict of interest

P.F. is the founder and CEO of Pharmahungary Group, a group of R&D companies. All other authors have nothing to disclose.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supporting information.

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