



HAL
open science

Hematopoietic Somatic Mosaicism Is Associated With an Increased Risk of Postoperative Atrial Fibrillation

Sandro Ninni, David Dombrowicz, Tanya Kuznetsova, Rocio Vicario, Vance Gao, Olivier Molendi-Coste, Joel Haas, Eloise Woitrain, Augustin Coisne, Annette E Neele, et al.

► **To cite this version:**

Sandro Ninni, David Dombrowicz, Tanya Kuznetsova, Rocio Vicario, Vance Gao, et al.. Hematopoietic Somatic Mosaicism Is Associated With an Increased Risk of Postoperative Atrial Fibrillation: Hematopoietic mosaicism predisposes to postoperative atrial fibrillation. *Journal of the American College of Cardiology*, 2023, 81 (13), pp.1263-1278. 10.1016/j.jacc.2023.01.036 . hal-04352926

HAL Id: hal-04352926

<https://hal.science/hal-04352926>

Submitted on 19 Dec 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Hematopoietic Somatic Mosaicism is Associated with an Increased Risk of Postoperative Atrial Fibrillation

Short title: Hematopoietic mosaicism predisposes to postoperative atrial fibrillation

Sandro Ninni MD, PhD^{a*}, David Dombrowicz PhD^{a*}, Tanya Kuznetsova PhD^{b+}, Rocio Vicario PhD^{c+}, Vance Gao PhD^a, Olivier Molendi-Coste PhD^{a+}, Joel Haas PhD^a, Eloise Woittrain PhD^a, Augustin Coisne MD, PhD^a, Annette E Neele PhD^b, Koen Prange PhD^b, Lisa Willemsen PhD^b, Samy Aghezzaf MD^a, Stamatina Frakogianni PhD^c, Amine Tazibet MD^a, Laurent Pineau PhD^a, James Robert White PhD^d, Jérôme Eeckhoutte PhD^a, Mohamed Koussa MD^a, Henri Dubrulle MD^a, Francis Juthier MD, PhD^a, Jérôme Soquet MD, PhD^a, André Vincentelli MD, PhD^a, Jean-Louis Edme MD, PhD^c, Menno de Winther^b, Frederic Geissmann MD, PhD^c, Bart Staels PhD^{a‡}, David Montaigne MD, PhD^{a‡}

* These authors equally contributed to this work

‡ Co-senior authors

- a. Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011-EGID, F-59000 Lille, France
- b. Experimental Vascular Biology, Department of Medical Biochemistry, Amsterdam Cardiovascular Sciences, Amsterdam Infection and Immunity, Amsterdam University Medical Centers, Amsterdam, The Netherlands
- c. Immunology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA
- d. Resphera Biosciences, Baltimore, MD, USA
- e. Univ. Lille, EA 4483, IMPECS: IMPact of Environmental ChemicalS on Human Health, CHU Lille, F-59000 Lille, France

Funding: This study was supported by grants from the Fédération Française de Cardiologie, the Fondation Leducq convention 16CVD01 “Defining and targeting epigenetic pathways in monocytes and macrophages that contribute to cardiovascular disease”, the European Genomic Institute for Diabetes (EGID, ANR-10-LABX-0046), the FRM (REFERENCE PROJET EQU202203014650) and the Agence Nationale de la Recherche (TOMIS leukocytes: ANR-CE14-0003-01). BS is a recipient of an Advanced ERC Grant (694717). RV was supported by the 2018 AACR-Bristol-Myers Squibb Fellowship for Young Investigators in Translational Immuno-oncology. Work at MSKCC is supported by MSKCC core grant (P30 CA008748) and NIH 1R01NS115715-01, 1 R01 HL138090-01, 1 R01 AI130345-01, and Basic and Translational Immunology Grants from Ludwig Center for Cancer Immunotherapy to FG, and Grant 18-40-15-VICA,T32- CA009512, MSKCC Precision Interception and Prevention Program, and Stand Up to Cancer Colorectal Cancer Dream Team Translational Research Grant to LAD. MdW is funded by grants from the Netherlands Heart Foundation (CVON: GENIUS2) and the Netherlands Heart Foundation and Spark-Holding BV (2019B016), AEN is a Dekker-fellow of the Netherlands Heart Foundation (2020T029).

Disclosures: JRW is founder and owner of Resphera Biosciences. FG has performed consulting for Third Rock venture. The remaining authors have nothing to disclose.

Corresponding author contact information: sandro.ninni@chu-lille.fr & david.montaigne@chu-lille.fr Department of Clinical Physiology and Echocardiography. University Hospital CHU Lille. Bd Jules Leclercq. 59037 Lille. France. +33 320 44 59 62.

Acknowledgements: The authors thank Bertrand ACCART and the Biological Resources Center of the Centre Hospitalier de Lille (BB 0033-00030) for handling and providing biological samples from the POMI-AF Study.

DATA AVAILABILITY:

RNA seq data are available at the following link:

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190353>

ABSTRACT

Background: On-pump cardiac surgery triggers sterile inflammation and post-operative complications such as atrial fibrillation (POAF). Hematopoietic somatic mosaicism (HSM) is a recently identified risk factor for cardiovascular diseases and results in a shift towards a chronic pro-inflammatory monocyte transcriptome/phenotype.

Objectives: To assess prevalence, characteristics, and impact of HSM on pre-operative blood and myocardial myeloid cells as well as on outcomes after cardiac surgery.

Methods: Blood DNA from 104 patients referred for surgical aortic valve replacement (AVR) was genotyped using the HemePACT panel (576 genes). Four screening methods were applied to assess HSM and post-operative outcomes were explored. In-depth blood and myocardial leukocyte phenotyping was performed in selected patients using mass cytometry and pre-and post-operative RNAseq analysis of classical monocytes.

Results: The prevalence of HSM in the patient cohort ranged from 29%, when considering the conventional HSM panel (97 genes) with variant allelic frequencies (VAF) \geq 2%, to 60% when considering the full HemePACT panel and VAF \geq 1%. Three out of 4 explored HSM definitions were significantly associated with higher risk for POAF. Based on the most inclusive definition, HSM carriers exhibited a 3.5 higher risk for POAF (age-adjusted OR (CI95%)=3.5(1.52-8.03);p=0.003) and an exaggerated inflammatory response following AVR. The pre-surgery myocardium of HSM carriers was enriched in activated CD64⁺CD14⁺CD16⁻ circulating monocytes and inflammatory monocyte-derived macrophages.

Conclusion: HSM is frequent in candidates for AVR, associated with an enrichment of pro-inflammatory cardiac monocyte-derived macrophages and predisposes to a higher incidence of POAF. HSM assessment may be useful in the personalized management of patients in the peri-operative period.

CONDENSED ABSTRACT

On-pump cardiac surgery triggers sterile local and systemic inflammation contributing to post-operative atrial fibrillation (POAF). Hematopoietic somatic mosaicism (HSM) is a recently identified risk factor for cardiovascular diseases. In a cohort of patients undergoing aortic valve replacement, the prevalence of HSM ranged from 29 to 60% according to applied screening method. Based on the most inclusive definition, HSM carriers exhibited a 3.5 higher risk for POAF and an increased post-operative inflammatory response. A pro-inflammatory immune cell profile in atrial myocardium and blood was observed in HSM carriers. HSM assessment may be useful in the personalized management of patients undergoing cardiac surgery.

Keywords: Post-operative atrial fibrillation; Cardiac surgery; Inflammation; Clonal hematopoiesis; Somatic mosaicism

ABBREVIATION LIST

AF: atrial fibrillation

AKI: acute kidney injury

AVR: aortic valve replacement

CABG: coronary artery by-pass graft

CHIP: clonal hematopoiesis of indeterminate potential

HSM: hematopoietic somatic mosaicism

LVEF: left ventricular ejection fraction

POAF: post-operative atrial fibrillation

SNV: single nucleotide variant

VAF: variant allelic frequency

Clinical trial: NCT#03376165

<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE190353>

INTRODUCTION

Cardiac surgery is among the most common major surgical procedures performed worldwide. Despite the emergence of trans-catheter interventions, such as trans-aortic valve replacement and percutaneous edge-to-edge trans-catheter mitral valve repair, the number of patients undergoing cardiac surgery is still significant because of the ageing population and the rising prevalence of associated co-morbidities ¹. These high-risk elderly patients are more prone to peri-operative complications such as acute hemodynamic instability, type 5 myocardial infarction (MI), post-operative atrial fibrillation (POAF) and acute kidney injury (AKI), resulting in worse clinical outcomes ^{2,3}.

On-pump cardiac surgery triggers sterile systemic and local innate inflammatory responses ⁴. This inflammatory response, which peaks 1-3 days after surgery, mainly stems from blood coming in direct contact with the cardiopulmonary bypass tubing system and from the organ reperfusion injury (e.g. the heart), leading to subsequent release of Danger-Associated Molecular Pattern molecules, complement activation and direct surgical trauma ⁵. Its intensity is highly variable among patients and remains unpredictable. Although evidence suggests that the peri-operative inflammation may be causally related to POAF, previous attempts to dampen the inflammatory response showed mitigated results on clinical outcomes ⁶.

Ageing is associated with accumulating post-zygotic mutations due to errors in the repair or replication of damaged DNA, leading to genetic heterogeneity within tissues called somatic mosaicism (SM) ⁷. This age-related SM has been largely characterized in circulating leukocytes with emphasis on genes previously found to be involved in leukemogenesis. Furthermore, low frequency mutations in a selection of HSM genes, termed “clonal hematopoiesis of indeterminate potential” (CHIP) genes, have been recently associated with adverse clinical outcomes such as coronary artery disease, chronic heart failure, incident atrial fibrillation and mortality following percutaneous aortic valve replacement ⁸⁻¹³. Importantly,

studies associating HSM to clinical outcomes used different screening methods with varying sequencing depth, gene panels and/or variant allelic frequency (VAF) thresholds, resulting in a significant heterogeneity in HSM definition (*i.e* gene number encompassed by the panel and VAF threshold). Although not completely understood, the mechanisms linking HSM to adverse cardiovascular outcomes are believed to result from a pro-inflammatory immune shift, which was mainly characterized in the context of DNMT3A and TET2 mutations^{14–16}. In this study, we assessed HSM frequency, defined using different criteria, in a population of patients undergoing cardiac surgery. We then determined whether HSM presence increases (pre-operative) myeloid cell activation and myocardial recruitment, and impacts the post-operative inflammatory response and outcomes after surgery.

METHODS

Cohort and pre-operative clinical assessment

The clinical cohort is part of the POMI-AF study (NCT#03376165), approved by the institutional ethics committee (Comité de Protection des Personnes Ile de France V). Written informed consent was obtained from all patients before inclusion.

Patients (aged ≥ 18 years) referred to the cardiovascular surgery department at the Lille University Hospital (Lille, France) for AVR (with or without coronary artery bypass graft) because of severe aortic valve stenosis. The day before surgery, the medical history of each patient was collected, and an electrocardiogram (ECG) recorded to detect potential existing atrial fibrillation (AF) at baseline.

A total of 442 patients candidate for cardiac surgery were included in the POMI-AF study from February 2017 to April 2019. Among 176 patients scheduled for aortic valve replacement, we excluded patients with a history of prior AF or who developed post-operative infection (defined as all clinical conditions leading to antibiotic prescription within the first week following surgery) to avoid infection-induced interference with HSM-dependent

inflammatory responses, resulting in a total of 133 patients (Figure S1). As specified in the statistical power calculation, the study was conducted until 52 patients presenting POAF were enrolled. Since POAF patients were globally older (Table S1), we selected 52 patients without POAF with a comparable distribution of predefined variables (age, BMI, diabetic status and gender). Further details regarding the patient selection process are available as supplementary materials. In these 104 selected patients, a large panel of 576 genes were genotyped for HSM (HemePACT panel, Table S2).

HSM screening strategies

To assess the correlation of HSM with age and clinical outcomes, 4 different definitions were adopted. These definitions are based upon different gene sets reported in previous studies which included: (i) HSM defined according to a list of 97 genes previously screened in studies assessing the association between CHIP and cardiovascular outcomes (conventional CHIP panel, Table S3)^{9,12,17}, (ii) HSM defined according to a large oncogenic panel including 576 genes previously associated with hematological malignancies, clonal hematopoiesis and solid tumors (HemePACT panel, Table S2).

For each gene set, according to previous studies, two VAF thresholds were assessed (2 and 1%) in order to evaluate the relevance of low VAF and its correlation with age and POAF.

Thus, 4 definitions for HSM were determined based on these 2 combined criteria:

- (i) Conventional CHIP panel, VAF \geq 2%
- (ii) Conventional CHIP panel, VAF \geq 1%
- (iii) HemePACT, VAF \geq 2%
- (iv) HemePACT, VAF \geq 1%

Prevalence of HSM was reported according to each definition, as well as its association with age and risk for POAF. HSM as defined by HemePACT, VAF \geq 1% was used as the reference for exploring its impact on pre- and post-operative immuno-inflammation.

Statistical analyses of clinical outcomes

Continuous variables were tested for normality with the Shapiro test. Continuous variables with Gaussian distribution are given as mean±SD. Continuous variables with non-Gaussian distribution are given as median (IQR). Categorical variables are given as percentages of individuals. Bivariate comparisons were performed using the t test for normally distributed continuous variables or the Mann-Whitney U test for not normally distributed variables. Bivariate comparisons of categorical variables were done with the χ^2 test.

A logistic regression backward model was used to explore predictors of POAF, AKI, type 5 MI, and included HSM status and pre-specified confounders previously associated with these outcomes (age, BMI, diabetes, and extracorporeal circulation duration). For the backward logistic regression model, candidate variables were removed when $p>0.2$. A backward multiple regression model was performed for post-operative CRP peak and post-operative troponin peak analysis and included HSM status, age, BMI, diabetes, extracorporeal circulation duration, and POAF. Normality for residuals of multiple regression models were assessed using the Shapiro-Wilk test. All analyses were done using MedCalc v16.4 (Olstead, Belgium).

Extended methods are provided as supplementary materials regarding post-operative outcomes assessment, sample size calculation, DNA sequencing, mass cytometry and RNAseq analysis.

RESULTS

HSM is highly prevalent in patients undergoing cardiac surgery.

HSM was assessed by deep targeted DNA sequencing of the leukocytes from 104 patients (52 POAF and 52 sinus rhythm patients) elected for AVR using a large panel of 576 genes (HemePACT panel, Table S2). The median age of the 104 patients was 70 years [66; 75], 36% were women and 29% had type 2 diabetes mellitus (T2D). As a result of the patients

selection process, POAF and sinus rhythm patients presented comparable distributions regarding age, BMI, diabetic status and gender (Table S1).

Analysis based on the 4 HSM definitions, differing in the number of included genes and VAF threshold (Figure 1A), resulted in an overall high prevalence of HSM ranging from 29 to 60% of patients (29% for the conventional CHIP panel with $\text{VAF} \geq 2\%$; 45% for conventional CHIP panel with $\text{VAF} \geq 1\%$; 44% for HemePACT panel with $\text{VAF} \geq 2\%$ and 60% for HemePACT panel with $\text{VAF} \geq 1\%$) (Figure 1B). Advanced age was positively associated with HSM irrespective of its definition (Figure 1C).

Using the HemePACT panel with $\text{VAF} \geq 1\%$, the most inclusive definition, most mutations consisted of single nucleotide variants (SNV) resulting in missense mutations (Figures S2A-B). As previously reported, the mutations frequently affected genes encoding epigenetic regulators and DNA damage response proteins, such as DNMT3A in 17% of patients (26% of mutations) and TET2 in 11% of patients (13% of mutations) (Figure S2C and Table S4). Additional mutations were found in several genes associated with the DNA damage response, such as ATM and SF3B1 (in 7% and 3% of patients) (Figure 1F). Thirty-two percent of patients presenting mutation had multiple mutated genes (Figure 1E). The VAFs ranged from 1.1 to 33.9% with a marked inter-gene heterogeneity (Figure S2D). Moreover, as previously shown, the number of mutations per patient increased with age (Figure S2E). This indicates that HSM is frequent in patients scheduled for cardiac surgery, which is in line with their elevated age.

HSM identifies patients at risk for POAF irrespective of other comorbidities.

HSM carriers, as defined by the HemePACT panel with $\text{VAF} \geq 1\%$, were older than non-HSM carriers, but did not display other significant differences regarding clinical pre-operative features (Table S5). Importantly, these HSM carriers had a 3.5 higher risk for POAF even after adjustment for age (age-adjusted OR (CI 95%)=3.5(1.52-8.03); $p=0.003$). This

association between HSM and POAF was found for all 4 HSM definitions (Figure 1D), albeit only borderline significant with the conventional CHIP panel with VAF \geq 2%.

The association of HSM with POAF onset was not only independent of age, but also of BMI, diabetes status and extracorporeal circulation duration (Table S6, S7). HSM status was not associated with the occurrence of other early complications such as AKI and type 5 MI.

HSM carriers display pre-operative alterations in peripheral blood monocytes and myocardial macrophages and an exacerbated post-operative inflammatory response.

Both HSM and POAF have been previously associated with inflammation, therefore the impact of HSM on post-operative inflammation and leukocytes was assessed.

Despite normal pre-operative blood CRP levels, HSM carriers presented a slightly higher pre-operative number of peripheral blood leukocytes due to an increase in monocytes (Table S5). Moreover, HSM carriers presented a higher inflammatory response following AVR, as measured by the post-operative CRP peak within 72 hours (148 ± 60 mg/l for non-HSM vs 200 ± 88 mg/l for HSM, $p=0.002$) (Figure 2A). The association of HSM with higher post-operative inflammation remained significant after adjustment for age, BMI, diabetes, and extracorporeal circulation duration. Interestingly, both POAF and HSM were independently associated with the postoperative CRP peak (Table S8).

To gain insight in the exacerbated inflammatory response following surgery and the increased POAF risk of HSM carriers, 2 subgroups of patients were selected for further detailed exploration of the pre-operative phenotype of peripheral blood and myocardial leukocytes. The immune phenotype of circulating leukocytes was assessed using in-depth unsupervised mass cytometry analysis of PBMC samples from 17 patients (11 HSM versus 6 non-HSM carriers) with myocardial tissue available (Table S9-S12). Unsupervised clustering revealed 37 clusters of peripheral blood leukocytes (Figure 2B&C) of which 3 were significantly higher in HSM patients (Figure 2D&E). Two clusters (1 and 11) contained CD14⁺CD16⁻

monocyte subsets expressing CD64/Fc γ RI α , the high affinity IgG receptor associated with activation/inflammation, and 1 cluster related to a CD4⁺ T cells subset (cluster 2) (Figure 2C). As HSM patients displayed alterations in pre-operative PBMC subsets (Figure 2E), including increased monocytes, as well as an increased post-operative inflammatory response (Figure 2A) and since classical monocytes are known to be associated with surgery induced-inflammation, RNA-seq transcriptomic analysis was performed on sorted pre-operative classical CD14⁺⁺CD16⁻ monocytes (Figure 3A) from 20 HSM and 13 non-HSM carriers (Table S13-S14). PCA analysis of differentially expressed genes identified 2 major clusters segregating most HSM carriers from non-carriers (Figure 3B). Volcano plot analysis showed that a larger proportion of genes was downregulated in monocytes from HSM carriers (down vs up: 327 vs 185 genes, based on FDR<0.1) (Figure 3C). None of the differentially expressed genes overtly related to classical inflammation, such as IL-1 β , IL-6 or TNF. Yet, pathway analysis revealed that several pathways were differentially expressed in HSM vs non-HSM carriers. These pathways corresponded to myeloid leukocyte activation, regulation of catabolic processes and autophagy, histone H3K4 demethylation, apoptosis processes, endosomal transport and TLR7 signaling (Figure 3D). The most significant changes were observed in the “Myeloid leukocyte activation” pathway, in which several upregulated genes showed interaction by gene correlation network analysis (Figure 3E). Interestingly several of these upregulated genes have been previously associated with cardiovascular or metabolic pathologies, such as TLR7, fibrinogen-like 2 (FGL2), phospholipase D1 (PLD1), anoctamine 6 (ANO6), vinculin (VLC), complement receptor 1 (CR1), PECAM1 and cathepsin S (CTSS) (Figure 2E). Interestingly, the expression of genes involved in monocyte proliferation and differentiation (CSF1R), recruitment (CCR2) or IgG-mediated activation (FCGR1a/CD64) was also up-regulated, while the expression of the nuclear receptor PPAR γ , involved in M2/anti-inflammatory macrophage polarization, was down-regulated (Figure 3F).

Taken together, these results indicate that inflammatory monocytes of HSM carriers display transcriptomic changes suggesting an enhanced (pre-operative) activation state with up-regulation of genes involved in proliferation, chemotaxis, cellular adhesion, and polarization. To gain insight into the activation state of the monocytes after surgery, a RNA-seq transcriptomic analysis was performed in paired samples of sorted classical monocytes taken from 3 HSM and 3 non-HSM patients before and 24 hours after surgery. A significant surgery effect was observed in all patients, which affected the innate immune response, cytokine signaling, regulation of apoptotic, and leukocyte activation pathways (Figure S3A). Although HSM and non-HSM patients shared common surgery-induced pathways (leukocyte cell-cell adhesion, chemical homeostasis, ion homeostasis, IL2-STAT5 signaling, regulation of dendritic cell antigen presentation and iron ion transmembrane transport pathways), some were specific to HSM patients, such as the MHC class II protein complex, positive regulation of cell adhesion, antigen processing and clathrin-coated endocytic vesicle membrane pathways (FigureS3B).

Interestingly, comparison of pre- and post-surgery transcriptomes assessed by PCA analysis revealed a lower Euclidian distance between pre- and post-surgery states in HSM patients compared to non-HSM patients (Figure 3H and Figure S3D-E), indicating smaller pre- to post-surgery differences in monocyte transcriptomes in HSM patients, indicative of the existence of a monocyte priming prior to surgery in HSM patients.

Since such an activation state might favor monocyte egress from the blood stream and myocardial recruitment, mass cytometry analysis of the myocardial myeloid leukocytes was performed in the 17 patients on whom the circulating leukocyte phenotype was previously analyzed (Figure 4A). As such, 16 myeloid clusters were identified (Figure 4B-C). Although HSM and non-HSM carriers presented similar clinical characteristics, the former displayed a distinct cardiac myeloid leukocyte population with an enrichment in cluster 4, which

corresponds to a subset of monocyte-derived macrophages (HLA-DR⁺CCR2⁺CD11c⁺CD64⁺CD206⁺) with high expression of CD64, a hallmark of inflammatory macrophages, and CD206, which has been associated with myocardial fibrosis¹⁸ (Figure 4D-F). Furthermore, this dysregulated macrophage cluster significantly correlated with 1 dysregulated circulating monocyte cluster (Figure 2C-D) identified in the HSM patients (Figure 4G).

Altogether, these data indicate that HSM carriers display an increase in a cluster of recruited monocyte-derived macrophages exhibiting an inflammatory phenotype in the myocardium, which correlates with clusters of circulating monocytes displaying similar inflammatory characteristics. These observations suggest a potential link between the circulating and tissue immune cell subsets.

DISCUSSION

Our results show that patients undergoing cardiac surgery display a high prevalence of HSM (as high as 60% with the most inclusive definition), which increases with age. HSM most frequently occurs in two genes (DNMT3A and TET2) encoding epigenetic regulators. Moreover, we found that the presence of HSM is associated with (i) increased circulating monocyte counts, specifically inflammatory CD64⁺ monocytes; (ii) circulating classical inflammatory monocyte gene profiles indicative of activation and chemotaxis; and (iii) an increase in monocyte-derived CD64⁺ inflammatory macrophages in the myocardium. In line, HSM carriers display a more pronounced peri-operative inflammatory response with a higher incidence of POAF independently of age and other confounders.

Our study highlights critical variations in HSM prevalence depending on the employed screening methods. The presence of HSM was initially identified as a major risk factor for coronary artery disease (CAD)⁹. When screening 74 predefined genes, these authors reported a prevalence of CHIP mutations of 17 and 10% in a cohort of elderly patients (median age of

70 years) with and without CAD, respectively, with VAFs ranging from 4 to 50% ⁹. Other studies, using deep-sequencing methodologies, reported a higher prevalence of HSM. For instance, a study on 20 healthy individuals ranging between 50 and 60 years, assessing a panel of 54 genes with variant detections as low as 0.003% VAF, identified a prevalence of 95% of TET2 and/or DNMT3A mutations ¹⁹. Thus, the high proportion of HSM observed in our cohort using the most inclusive definition is likely due to both the depth of the sequencing allowing the identification of variants with >1% VAF, as well as the large number of screened genes. Interestingly, our approach allowed the identification of mutations in genes playing roles in the DNA-damage response, such as the Ataxia Telangiectasia Mutated (ATM) ²⁰ or the Splicing Factor 3B subunit 1 (SF3B1) ²¹. Although not directly involved in epigenetic regulation, mutations in genes related to the DNA-damage response might induce a pro-inflammatory ‘senescence-associated secretory’ phenotype ²².

Although a few studies investigated a functional link between cardiovascular phenotypes and specific mutations involving TET2 ²³, JAK2 ¹⁶ or DNMT3A ²⁴ in animal models, other studies highlighted that HSM might result from a chronic inflammation, leading to increased myeloid turnover and subsequent mutations ²⁵. Thus, the link between HSM and cardiac pathologies is probably a complex process involving both non-HSM and HSM related inflammation. Furthermore, the diversity of mutations/mosaicism and their various mechanistic effects (e.g: loss of Y chromosome leading to a pro-fibrotic phenotype involving the TGF β pathway ²⁶, TET2 mutations involving IL-1 β pathway) render this pathophysiological model highly complex. Interestingly, we did not identify transcriptome signatures associated with IL1- β pathway alteration when pooling different mutation subtypes. This lack of association might be related to the fact that IL-1 β pathway is not statistically dominant compared to pathways involved in inflammation.

We observed a pre-operative increase in specific circulating inflammatory monocytes subsets, which correlated with an increase in myocardial inflammatory macrophages. In line, among the transcriptomic alterations found in inflammatory monocytes, the expression of several genes related to activation pathways, involved in the response of monocytes to pro-inflammatory or chemotactic stimuli, were elevated. Consistent with an increased number of pro-inflammatory macrophages in the myocardium, we observed an increased inflammatory response in HSM carriers following surgery associated with a higher incidence of POAF.

POAF occurs when transient post-operative triggers act on a vulnerable atrial substrate produced by pre-operative, surgery-induced and post-operative remodeling processes². Taken together, our data suggest that HSM could help identifying patients at risk for (i) a higher inflammatory response resulting in a higher post-operative trigger driven by monocyte activation, and (ii) a higher atrial vulnerability resulting from an immune cell population shift within the atrial myocardium. HSM carrier patients may, as such, be good candidates for anti-inflammatory drugs to prevent “disproportionate” post-operative inflammation and POAF occurrence.

Limitations

This clinical study cannot formally establish a causal link between the presence of HSM, pre-operative alterations of monocyte/macrophage populations and post-operative inflammation and complications. This exploratory study was designed to be hypothesis generating regarding HSM and POAF, and requires additional confirmation and mechanistic studies. Furthermore, our study provides a basis to elaborate further studies and to more accurately estimate the sample size needed to correlate HSM and POAF outcomes using alternative screening methods. Although the selected patients display similar distribution profiles regarding several clinical variables, the selection process did not involve the use of propensity scores. The current study did not allow to explore specifically the effect of high clone size on POAF since

the number of patients presenting high VAFs was low. Such association should be explored by additional studies.

CONCLUSION

HSM is frequent in patients undergoing cardiac surgery. Its prevalence critically depends on the gene number included in the definition with the broadest identifying patients at very high risk for POAF following surgery. This increased risk for POAF could be related to an increased post-operative inflammatory response driven by primed monocytes and increased atrial vulnerability driven by an immune shift in the atrial myocardium (central illustration). Assessment of HSM and/or its related alterations in monocytes might thus be valuable indicators to personalize management in the context of cardiac surgery.

PERSPECTIVES

Competency in Medical Knowledge 1: On-pump cardiac surgery triggers sterile local and systemic inflammation and post-operative complications such as atrial fibrillation. Identification of patients at risk for exacerbated inflammatory response after cardiac surgery might improve postoperative outcomes.

Competency in Medical Knowledge 2: Age-related hematopoietic somatic mosaicism (HSM) has been previously associated with adverse clinical outcomes such as coronary artery disease, chronic heart failure and mortality following percutaneous aortic valve replacement in patients without hematological disease. Although not completely understood, the mechanisms linking HSM to adverse cardiovascular outcomes are believed to result from a pro-inflammatory immune shift. Such immune shift might play a critical role in inflammatory response following cardiac surgery.

Translational Outlook 1: Patients undergoing cardiac surgery display a high prevalence of HSM (as high as 60% with the most inclusive definition), which increases with age. HSM carriers display an increased circulating monocyte count, specifically inflammatory CD64⁺ monocytes; circulating classical inflammatory monocyte gene profiles indicative of activation and chemotaxis; and an increase in monocyte-derived CD64⁺ inflammatory macrophages in the myocardium.

Translational Outlook 2: In line, HSM carriers display a more pronounced peri-operative inflammatory response with a 3.5-fold higher incidence of POAF independently of age and other confounders. Identification of HSM carriers may be useful in the personalized management of patients in the peri-operative period

REFERENCES

1. Englum BR, Ganapathi AM, Schechter MA, Harrison JK, Glower DD, Hughes GC. Changes in Risk Profile and Outcomes of Patients Undergoing Surgical Aortic Valve Replacement From the Pre- to Post-Transcatheter Aortic Valve Replacement Eras. *Ann Thorac Surg.* 2016;101:110–117.
2. Dobrev D, Aguilar M, Heijman J, Guichard J-B, Nattel S. Postoperative atrial fibrillation: mechanisms, manifestations and management. *Nat Rev Cardiol.* 2019;16:417–436.
3. Wang Y, Bellomo R. Cardiac surgery-associated acute kidney injury: risk factors, pathophysiology and treatment. *Nat Rev Nephrol.* 2017;13:697–711.
4. Wan S, Marchant A, DeSmet JM, et al. Human cytokine responses to cardiac transplantation and coronary artery bypass grafting. *J Thorac Cardiovasc Surg.* 1996;111:469–477.
5. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol.* 2020;20:95–112.
6. Imazio M, Brucato A, Ferrazzi P, et al. Colchicine for prevention of postpericardiotomy syndrome and postoperative atrial fibrillation: the COPPS-2 randomized clinical trial. *JAMA.* 2014;312:1016–1023.
7. Vijg J, Dong X. Pathogenic mechanisms of somatic mutation and genome mosaicism in aging. *Cell.* 2020;182:12–23.
8. Jaiswal S, Libby P. Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease. *Nat Rev Cardiol.* 2020;17:137–144.
9. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med.* 2017;377:111–121.

10. Mas-Peiro S, Hoffmann J, Fichtlscherer S, et al. Clonal haematopoiesis in patients with degenerative aortic valve stenosis undergoing transcatheter aortic valve implantation. *Eur Heart J*. 2020;41:933–939.
11. Yu B, Roberts MB, Raffield LM, Zekavat SM, et al. Association of Clonal Hematopoiesis With Incident Heart Failure. *J Am Coll Cardiol*. 2021;78:42–52.
12. Pascual-Figal DA, Bayes-Genis A, Díez-Díez M, et al. Clonal Hematopoiesis and Risk of Progression of Heart Failure With Reduced Left Ventricular Ejection Fraction. *J Am Coll Cardiol*. 2021;77:1747–1759.
13. Kar SP, Quiros PM, Gu M, et al. Genome-wide analyses of 200,453 individuals yield new insights into the causes and consequences of clonal hematopoiesis. *Nat Genet*. 2022;54:1155–1166.
14. Abplanalp WT, Cremer S, John D, et al. Clonal Hematopoiesis-Driver DNMT3A Mutations Alter Immune Cells in Heart Failure. *Circ Res*. 2021;128:216–228.
15. Abplanalp WT, Mas-Peiro S, Cremer S, John D, Dimmeler S, Zeiher AM. Association of Clonal Hematopoiesis of Indeterminate Potential With Inflammatory Gene Expression in Patients With Severe Degenerative Aortic Valve Stenosis or Chronic Postischemic Heart Failure. *JAMA Cardiol*. 2020;5:1170–1175.
16. Fidler TP, Xue C, Yalcinkaya M, et al. The AIM2 inflammasome exacerbates atherosclerosis in clonal haematopoiesis. *Nature*. 2021;1–6.
17. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371:2488–2498.
18. Ma Y, Mouton AJ, Lindsey ML. Cardiac macrophage biology in the steady-state heart, the aging heart, and following myocardial infarction. *Transl Res*. 2018;191:15–28.
19. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat Comm*. 2016;7:12484.

20. Blackford AN, Jackson SP. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. *Mol Cell*. 2017;66:801–817.
21. Singh S, Ahmed D, Dolatshad H, et al. SF3B1 mutations induce R-loop accumulation and DNA damage in MDS and leukemia cells with therapeutic implications. *Leukemia*. 2020;34:2525–2530.
22. Zhang B, Fu D, Xu Q, et al. The senescence-associated secretory phenotype is potentiated by feedforward regulatory mechanisms involving Zscan4 and TAK1. *Nat Comm*. 2018;9:1723.
23. Sano S, Oshima K, Wang Y, et al. Tet2-mediated Clonal Hematopoiesis Accelerates Heart Failure through a Mechanism Involving the IL-1 β /NLRP3 Inflammasome. *J Am Coll Cardiol*. 2018;71:875–886.
24. Rauch PJ, Silver AJ, Gopakumar J, et al. Loss-of-Function Mutations in Dnmt3a and Tet2 Lead to Accelerated Atherosclerosis and Convergent Macrophage Phenotypes in Mice. *Blood*. 2018;132:745.
25. Heyde A, Rohde D, McAlpine CS, et al. Increased stem cell proliferation in atherosclerosis accelerates clonal hematopoiesis. *Cell*. 2021;184:1348-1361.e22.
26. Sano S, Horitani K, Ogawa H, et al. Hematopoietic loss of Y chromosome leads to cardiac fibrosis and heart failure mortality. *Science*. 2022;377:292–297.

Figure legends:

Figure 1. Prevalence of HSM according to screening strategies and association with POAF

A. Four screening strategies were applied to detect HSM: conventional CHIP panel VAF $\geq 2\%$; conventional CHIP panel VAF $\geq 1\%$; HemePACT panel VAF $\geq 2\%$ and HemePACT panel VAF $\geq 1\%$. **B.** Prevalence of HSM according to each screening method. **C.** Prevalence of HSM according to age and screening method. †:Chi square for trend. **D.** Association between POAF and HSM according to screening method. **E.** Proportion of patients presenting multiple mutation among mutation carriers. **F.** Venn diagram showing the mutated genes in SR and POAF patients. Numbers in brackets indicate the number of patients carrying at least one mutation for each gene. For genes mutated in both SR and POAF patients, numbers in brackets are (number of SR patients carrying at least one mutation / number of POAF patients carrying at least one mutation) for each gene.

Figure 2. Postoperative inflammatory response and pre-operative high dimensional PBMC immune phenotype

A. postoperative CRP peak (within 72 hours) according to HSM **B.** unsupervised clustering map of PBMC cells with general populations and **C.** 37 individual clusters were identified **D.** Heatmap representing characteristics of each identified cluster regarding the intensity of expression of each marker. **E.** Volcano plot representing differences in each cluster according to HSM status **F.** Violin plots representing significantly dysregulated clusters according to HSM. Cluster 1: 62432 [30600; 168688] cells/ml for non-HSM vs 148535 [117895; 211597] cells/ml for HSM (FDR<0.001); cluster 2: 62197 [54074; 122329] cells/ml for non-HSM vs 130048 [52188; 259142] cells/ml for HSM (FDR<0.001); cluster 11: 39904 [28766; 71982] cells/ml for non-HSM vs 96683 [63700; 149464] cells/ml for HSM (FDR=0.04).

Hematopoietic somatic mosaicism (HSM) as defined by the HemePACT panel with VAF upper than 1%.

Figure 3. Pre-operative transcriptome phenotype of peripheral blood monocytes

A. Classical monocytes sorting **B.** Principal component analysis (PCA) plot, using log-normalized expression values of top-expressing 10,000 genes. **C.** Volcano plot of the top-expressing 10,000 genes. "Myeloid leukocyte activation" genes in red. Dashed line indicates FDR=0.1. **D.** Biological Process terms most affected by HSM, as ranked by consensus p-value. Terms with more than 75% of member genes in the 10,000 most-expressed genes set were considered. **E.** Correlation network of top 70 significant genes in "Myeloid leukocyte activation" term. Edges connect genes whose partial correlation adjusted for HSM has Pearson's $r > 0.8$ **F.** Violin plots of selected gene. TLR7($p=0.002$;FDR=0.07; $\log_2FC=1.22$); Cathepsin C ($p=0.0002$;FDR=0.059; $\log_2FC=0.77$); Cathepsin S($p=0,004$;FDR=0,09; $\log_2FC=0.56$); CCR2($p=0.01$;FDR=0.11; $\log_2FC=1.09$); PECAM1($p=0.001$;FDR=0.07; $\log_2FC=0.62$); Vinculin($p=0.001$;FDR=0.07; $\log_2FC=0.63$); CR1($p=0,004$;FDR=0,09; $\log_2FC=0.70$); CSF1R($p=0.009$;FDR=0.11; $\log_2FC=0.85$); FCGR1A($p=0.02$; $\log_2FC=0.58$); PPAR G($p=0.003$;FDR=0.08; $\log_2FC=-2.12$). **G.** Surgery-induced transcriptome explored in PBMCs obtained before and 48h after surgery. **H.** Euclidian distance extrapolated from PCA between transcriptome status of HSM and non-HSM patients, before and after surgery.

Figure 4. Myocardial immune phenotype according to HSM status

A. Right appendage biopsies were collected for 17 patients and leukocyte fraction was isolated and characterized using mass cytometry with unbiased approach based on clustering. **B-C.** unsupervised clustering map of myeloid cells with general populations (**upper panel**) and 16 clusters (**lower panel**) re-identified. **D.** Heatmap representing characteristics of each identified cluster regarding the intensity of each marker. **E.** Violin plots representing the

proportion of each cluster among the total CD45⁺ cells in myocardium according to HSM status. Computations assumed same scatter for subsets (*cluster 4: 4.9±3.2% vs 8.5±3.4% of CD45⁺ cells, FDR<0.001). **F.** Relative expression of markers defining cluster 4 in the map. Dashed line represents cluster 4 on each map. **G.** Correlation between atrial tissue (AT) cluster 4 and PBMCs cluster 11 (monocytes) upregulated in HSM patients. Hematopoietic somatic mosaicism (HSM) as defined by the HemePACT panel with VAF≥1%.

Central illustration. Association between HSM and POAF

Ageing leads to accumulating post zygotic mutations in bone marrow hematopoietic stem cells. These mutations are associated to increased monocyte activation in peripheral blood and monocytes derived macrophages in myocardium. As a result of the surgery, an increased inflammatory response is observed in HSM carriers. Such increase in inflammatory response, combined to myocardial macrophages infiltration could lead to the increase in POAF incidence observed in HSM carriers.