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RESEARCH ARTICLE

Espinosa-Bautista et al: Menopause and T-helper phenotypes

Influence of menopause status on T-helper cell profiles in acute myocardial infarction

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ABSTRACT

Estrogens modulate immune responses, particularly the activation and polarization of CD4+ T cells, which play key roles in cardiovascular homeostasis. This proof-of-concept study investigated the effect of menopausal status on the polarization of T-helper (Th) cells in women with acute myocardial infarction (AMI). A total of 41 female AMI patients were enrolled—7 premenopausal and 34 postmenopausal—and compared with a group of 17 male AMI patients. Flow cytometry was used to evaluate CD4+ T-cell subsets, including Th1 (T-bet+), Th2 (GATA3+), and Th17 (RORγt+) phenotypes. Serum levels of representative cytokines were also measured. Women exhibited higher numbers of circulating CD4+ T cells compared to men, with a marked shift toward the Th1 phenotype. Postmenopausal women demonstrated increased cardiovascular risk, as indicated by higher QRISK3 and GRACE scores, as well as elevated levels of C-reactive protein and cardiac troponin T compared to premenopausal women. However, menopausal status had minimal impact on Th cell polarization, as no significant differences were observed in the proportions of Th1, Th2, or Th17 subsets between premenopausal and postmenopausal women. Similarly, levels of interleukin-6, interleukin-1β, interleukin-10, tumor necrosis factor, and monocyte chemoattractant protein-1 were comparable between the two groups. This proof-of-concept study highlights sex-specific differences in immune responses and inflammatory profiles during AMI. Women exhibited a stronger polarization toward the Th1 phenotype, along with elevated markers of inflammation and myocardial injury. Notably, menopausal status did not significantly affect lymphocyte subpopulations or circulating cytokine levels.

Keywords: Myocardial infarction; T-helper cells; menopause; inflammation.

INTRODUCTION

Coronary artery disease (CAD) remains a leading global health concern, with acute myocardial infarction (AMI) representing its most severe manifestation [1]. Epidemiological data reveal significant sex-based differences in CAD prevalence, with premenopausal women exhibiting approximately half the risk compared to men [2]. This disparity diminishes after menopause, likely due to the loss of estrogen's cardioprotective effects [2-4]. In women, regional fat accumulation—particularly in breast tissue—has been associated with increased cardiovascular risk, even before menopause, through the release of pro-inflammatory cytokines and activation of pro-apoptotic pathways (5). These processes contribute to myocardial injury and impaired cardiac function. Chronic inflammation plays a central role in the pathogenesis of AMI and serves as a negative diagnostic, prognostic, and monitoring marker in CAD (6).

Myocardial ischemia and necrosis trigger a rapid inflammatory response that is essential for healing, with $CD4^+$ T-helper (Th) cells playing a major role through their functional polarization [7]. In male patients with AMI, studies have consistently reported a dysregulated Th cell profile marked by a Th1/Th2 imbalance and increased Th17 responses [8,9]. Estrogens modulate T-cell function through genomic mechanisms via estrogen receptors (ERα and ERβ), which bind to estrogen response elements in the promoter regions of target genes [7]. These effects are complemented by non-genomic signaling pathways involving mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) activation. Through these mechanisms, estrogens promote Th2-skewed responses by enhancing interleukin (IL)-4 and IL-10 production, while suppressing Th1-associated cytokines such as interferon-gamma. Conversely, AMI has been associated with increased peripheral Th17 cells and Th17-related cytokines (IL-17, IL-6 and IL-23), alongside reductions in regulatory T (Treg) cells and their associated cytokines, including IL-10 and transforming growth factor-beta [9]. Despite this knowledge, data on Th cell polarization in women with AMI remain limited, particularly in relation to menopausal status and the immunomodulatory effects of estrogens.

This proof-of-concept study aimed to investigate the polarization of Th1, Th2, and Th17 subsets in $CD4^+$ T cells from women with AMI, with a special focus on the differences between premenopausal and postmenopausal individuals.

MATERIALS AND METHODS

Study design

This single-center study enrolled 41 adult women admitted to the Coronary Care Unit for AMI, as defined by the Fourth Universal Definition of Myocardial Infarction [10], between January 2022 and April 2023. Our hospital is a specialized university clinical center dedicated to cardiovascular diseases. The diagnosis of AMI was established based on the presence of anginal symptoms coupled with evidence of myocardial injury, as indicated by elevated cardiac troponin T (cTnT) or creatine kinase MB isoenzyme (CK-MB) levels, with at least one measurement exceeding the 99th percentile upper reference limit. Exclusion criteria included ischemic symptoms lasting > 72 hours, pregnancy, active infections, neoplasia, autoimmune or inflammatory disorders, blood dyscrasias, recent surgery, or end-stage kidney or heart disease. Eleven men were included as a comparison group and met the same criteria, except for sex-specific exclusions.

Natural menopause was defined as the absence of menstruation for at least 12 consecutive months, in the absence of medical or pathological causes [11]. Women receiving hormone replacement therapy (HRT) or hormonal contraceptives were excluded.

Clinical assessments

Upon hospital admission, demographic and clinical data were collected, including the QRISK3 score, a validated algorithm for estimating the 10-year risk of developing CAD, and the GRACE score, a tool for stratifying the risk of in-hospital and post-discharge mortality [12,13]. Coronary angiography was performed in the hemodynamics laboratory and analyzed by interventional cardiologists from our institution. Significant CAD was defined as > 50% luminal narrowing in a major coronary artery or the left main coronary artery. Patients were followed daily until discharge, and a composite outcome of major adverse cardiac events (MACE) was scored, including acute heart failure, pulmonary edema, recurrent myocardial infarction, cardiogenic shock, or death.

Flow cytometry

Blood samples were collected within 60 minutes of admission to the Coronary Care Unit. Peripheral blood mononuclear cells (PBMCs) were isolated and analyzed using a FACSAria flow cytometer (BD Biosciences; San Jose, CA, USA). CD4$^+$ cells were identified using antibodies targeting CD4 (PercP; BioLegend; San Diego, CA, USA), T-bet (FITC; Th1

phenotype), GATA3 (PE; Th2 phenotype), and RORγt (APC; Th17 phenotype) (eBioscience; San Diego, CA, USA). Flow cytometry techniques and gating strategies were performed as previously described [14]. Assays were conducted by a single operator within 6 hours of blood collection to ensure sample integrity, following best-practice recommendations [15]. Intra-assay variability ranged from 0.7% (GATA3-PE) to 7.7% (RORγt-APC).

Cytokine measurement

Serum levels of prototypical inflammatory (IL-6, IL-1beta, tumor necrosis factor [TNF], and monocyte chemoattractant protein-1 [MCP-1/CCL2]) and anti-inflammatory (IL-10) cytokines were measured in duplicate using enzyme-linked immunosorbent assays (ELISA; BioLegend). Serum samples were available exclusively from female participants, as only ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples were collected from male patients.

Ethical statement

The study was approved by the local ethics committee (protocol codes 18-1089 and 21-1273) and adhered to the principles outlined in the Declaration of Helsinki and relevant local regulations. Written informed consent was obtained from all patients for the use of their clinical data and blood samples for research purposes. Medical interventions were administered solely at the discretion of treating physicians, and the study did not influence patient treatment or clinical decisions.

Statistical analysis

Data distribution was evaluated using the Shapiro-Wilk test. Categorical variables were presented as percentages and analyzed using Fisher's exact test. Continuous data were described as medians with minimum-maximum ranges and compared using the Mann-Whitney U test. Correlation analyses were performed using Spearman's ρ coefficient, with 95% confidence intervals. All analyses were two-tailed, and statistical significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad Prism v.9.3.1 (GraphPad Software; La Jolla, CA, USA).

RESULTS

The study included 41 women (median age: 55 years; range: 24-68), comprising 7 premenopausal and 34 postmenopausal participants, along with a comparison group of 17 men (median age: 65 years; $p = 0.158$). Two women who had experienced amenorrhea for more

than 12 but less than 36 months (borderline perimenopause) were classified as postmenopausal. While demographic characteristics were comparable (Table 1), men exhibited higher QRISK3 scores (13.7% vs. 9.0%; p = 0.037) and creatinine levels (1.0 vs. 0.7 mg/dL; p < 0.001). In contrast, women showed higher levels of cTnT (1086 vs. 196 pg/mL; p = 0.025) and high-sensitivity C-reactive protein (hsCRP; 5.3 vs. 2.6 mg/L; p = 0.038).

Among women, postmenopausal participants were older (57 vs. 46 years; p < 0.001) and had a higher cardiovascular risk (QRISK3: 9.5% vs. 3.0%; p = 0.018) compared to premenopausal women. No differences were observed in AMI type (ST-elevation AMI: 85% vs. 70%; p = 0.651), in-hospital therapies, or outcomes, including MACE (23.5% vs. 42.8%; p = 0.360) and mortality (2.9% vs. 0; p > 0.999) rates. However, postmenopausal women exhibited a higher risk profile, as reflected as a greater probability of in-hospital mortality (0.8% vs. 0.3%) according with the GRACE score (100 vs. 76 points; p = 0.004).

Flow cytometry results (Table 2) showed that women had higher $CD4^+$ cell counts compared to men (3555 vs. 2801 per 10000 PBMCs; p = 0.003). Th1 polarization tended to be more pronounced in women compared to men (32.7% vs. 28.8%), along with a higher Th1/Th2 ratio (1.5 vs. 1.1; p = 0.081). Th17 proportions were similar between sexes. When comparing premenopausal and postmenopausal women, the number of circulating $CD4^+$ cells was similar (3555 vs. 3611 per 10000 PBMCs; p = 0.622). Premenopausal women demonstrated a slight predominance of cells polarized towards the Th17 phenotype (55.0% vs. 42.2%; p = 0.266), whereas postmenopausal women exhibited a predominance towards Th1 (33.9% vs. 28.5%; p = 0.125) and Th2 (19.2% vs. 16.8%; p = 0.799) phenotypes. However, Th1/Th2 balance remained similar between the two groups (1.5 vs. 1.5; p = 0.773). In stratified analyses, total $CD4^+$ T cell counts were significantly lower in men compared to both premenopausal (p = 0.023) and postmenopausal (p = 0.007) women. However, no significant differences were observed in polarization profiles or in the Th1/Th2 ratio across groups.

No significant differences were found in serum levels of MCP-1/CCL-2 (312 vs. 234 pg/mL; p = 0.212), IL-6 (20 vs. 38 pg/mL; p = 0.544), TNF (20 vs. 7 pg/mL; p = 0.662), IL-1beta (2.3 vs. 5.1 pg/mL; p = 0.306), or IL-10 (3.9 vs. 3.9 pg/mL; p = 0.409) between premenopausal and postmenopausal women. Additionally, cTnT levels showed no significant correlations with hsCRP (ρ = 0.18, -0.14 to 0.47), IL-6 (ρ = -0.14, -0.45 to 0.20), IL-1beta (ρ = -0.16, 00.47 to 0.17), TNF (ρ = 0.11, -0.23 to 0.42), or IL-10 (ρ = 0.08, -0.25 to 0.40).

DISCUSSION

This study aimed to investigate sex- and menopausal status-based differences in lymphocyte phenotypes among patients with AMI. Our findings revealed that women exhibited higher numbers of circulating $CD4^+$ cells compared to men, with a distinct polarization toward the Th1 phenotype. Intriguingly, menopausal status appeared to exert no significant influence on $CD4^+$ cell subpopulations or levels of circulating inflammatory mediators, suggesting that other factors may play a dominant role during the early phases of the AMI.

The polarization of $CD4^+$ cells is heavily influenced by both inflammatory signals and hormonal environment [16]. Estrogens are critical regulators of this plasticity, with lower levels favoring pro-inflammatory subsets (e.g., Th1 and Th17) and higher levels promoting anti-inflammatory phenotypes (e.g., Th2 and Tregs). This adaptability is essential in the AMI-associated healing process, where pro-inflammatory subsets contribute to debris clearance, while anti-inflammatory subsets facilitate tissue repair and fibrosis [7]. Tregs are important for controlling excessive inflammation and promoting cardiac healing, with implications for post-AMI outcomes and ventricular remodeling [17-18]. Evidence suggests that women, particularly premenopausal women, may exhibit stronger Treg responses than men, potentially reflecting the immunomodulatory effects of estrogen and progesterone [16]. In our cohort, however, women displayed greater Th1 polarization and a higher Th1/Th2 ratio than men, aligning with previous findings that associate Th1-dominant responses with adverse cardiac outcomes [19]. Interestingly, we observed no significant differences in T cell polarization between premenopausal and postmenopausal women, although Tregs were not evaluated. This raises the possibility that the acute inflammatory milieu of AMI may override hormonal modulation, or that similar clinical and angiographic characteristics of CAD across these groups may mask subtle hormonal effects.

Another key observation is that women demonstrated higher levels of hsCRP and cTnT compared to men, despite having a lower baseline cardiovascular risk. This finding aligns with previous studies showing elevated baseline hsCRP levels in women, particularly among certain populations, such as African Americans [20,21]. Elevated hsCRP levels in women have been showed to predict cardiovascular disease independently of lipid levels and are strongly associated with increased in-hospital mortality during AMI [21-23]. Although hsCRP levels were similar between premenopausal and postmenopausal women in our study, postmenopausal women exhibited significantly higher cardiovascular risk, approaching levels observed in men. Regarding cTnT, while baseline concentrations are typically higher in men,

cTnT levels are more predictive of cardiovascular events in women [24]. This discrepancy may be related to differences in cardiac mass, the prevalence of subclinical CAD, and the protective effects of estrogens, which influence atherosclerosis risk factors, thrombus formation, vasoreactivity, and vascular apoptosis [25,26].

Our observation of an elevated cardiovascular risk profile in postmenopausal women aligns with previous epidemiological evidence suggesting a convergence in the incidence of CAD between sexes after menopause [2,3]. Studies of cardiovascular risk factors in premenopausal women have identified dyslipidemia, excessive smoking, hypertension, and diabetes as critical contributors [27,28]. These factors were prevalent in our study population and were notably overrepresented in postmenopausal women. Angiographic studies indicate distinct patterns of CAD presentation depending on menopausal status, with premenopausal women typically exhibiting single-vessel involvement, particularly in the left anterior descending artery [29,30]. Our findings suggest that postmenopausal women have a higher atherosclerotic burden and greater CAD severity, which emphasizes the importance of incorporating menopausal status into risk stratification and management strategies for women presenting with CAD [31].

Although total or near-total coronary occlusion is the most common angiographic finding in STEMI, it is less frequently observed in other forms of AMI. In our cohort, about one-quarter of the patients did not present with STEMI, and several exhibited obstructive lesions involving less than 50% of the vascular lumen. Notably, two patients met criteria for AMI with non-obstructive coronary arteries (MINOCA). In addition, most patients had received pharmacological reperfusion therapy prior to angiography, suggesting that some nonsignificant occlusions may reflect partial recanalization following fibrinolysis. Importantly, no differences in leukocyte subpopulations were observed between patients with vascular occlusions above or below the 50% threshold (data not shown).

Although premature ovarian failure increases cardiovascular risks, HRT has not been shown to mitigate this risk, regardless of the timing or duration of therapy [32,33]. Furthermore, findings from the Women's Health Initiative suggest a modest but significant increase in CAD risk in postmenopausal women receiving long-term HRT [34]. In contrast, emerging evidence suggests metformin may modulate inflammation involved in CAD pathogenesis and improve clinical outcomes in AMI patients [35].

We acknowledge that the small sample size may have limited our ability to detect subtle differences in $CD4^+$ T cell subpopulations based on menopausal status. The low number of

premenopausal women enrolled reflects the rarity of AMI in this demographic, which, conversely, highlights the unique value of the data obtained. A further limitation is the availability of only a single-time-point cTnT measurement, precluding longitudinal analysis of cytokine-to-troponin correlations. Additionally, sex hormone levels and ovarian reserve markers (e.g., anti-Müllerian hormone or ultrasound-based assessments) were not obtained. While these would have strengthened our mechanistic interpretation, their measurement was constrained by the urgency of patient stabilization and the need to minimize blood sampling. Moreover, acute stress-related hormonal fluctuations may limit the interpretation of endocrine data in this setting. Finally, body composition data (e.g., body mass index, waist-to-hip ratio, or breast fat metrics) were not collected, potentially confounding cytokine profiles, as adiposity can heighten systemic inflammation. These considerations should be addressed in future prospectively designed studies.

CONCLUSION

This proof-of-concept study highlights sex-specific differences in immune responses and inflammatory profiles during AMI. Women demonstrated pronounced polarization toward the Th1 phenotype, along with elevated inflammatory and myocardial lysis markers. Interestingly, menopausal status did not appear to significantly influence lymphocyte subpopulations or cytokine levels; however, further studies are necessary to confirm these preliminary findings.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Main characteristics of the study participants

	Participants grouped by sex		p	Women grouped by menopausal status		p
	Men (n = 17)	**Women (n = 41)**		**Pre (n = 7)**	**Post (n = 34)**	
Age, in years	65 (45-81)	55 (24-68)	0.158	46 (24-52)	57 (47-68)	**< 0.001**
Hypertension, n (%)	7 (41)	24 (58)	0.260	2 (28)	22 (64)	0.105
Diabetes, n (%)	8 (47)	24 (58)	0.563	3 (42)	21 (61)	0.421
Smoking, n (%)	11 (64)	21 (51)	0.397	2 (28)	19 (55)	0.237
Previous MI, n (%)	7 (41)	8 (19)	0.107	0	8 (23)	0.310
QRISK3 score, %	13.7 (3.0-50.4)	9.0 (0.1-28.2)	**0.037**	3.0 (0.1-13.1)	9.5 (1.2-28.2)	**0.018**
Laboratory data at hospital admission						
• hsCRP, mg/L	2.6 (0.2-67.7)	5.3 (0.4-239.0)	**0.038**	5.0 (1.0-8.2)	5.5 (0.4-239.0)	0.094
• Glucose, mg/dL	144 (91-355)	161 (83-428)	0.500	132 (91-340)	165 (83-428)	0.392
• Triglycerides, mg/dL	126 (63-304)	150 (54-449)	0.162	125 (68-413)	159 (54-449)	0.256
• Total cholesterol, mg/dL	160 (95-276)	166 (81-252)	0.229	147 (81-218)	176 (93-252)	0.208
• LDL-C, mg/dL	97 (34-222)	96 (29-177)	0.587	95 (43-146)	99 (29-177)	0.697
• HDL-C, mg/dL	37 (27-49)	39 (27-84)	0.141	31 (27-49)	40 (27-84)	0.118
• Hemoglobin, g/dL	16 (11-19)	14 (9-16)	**< 0.001**	13 (11-15)	14 (9-16)	0.131
• Leukocytes $x10^3/mm^3$	10.5 (7.3-13.8)	11.6 (5.8-18.3)	0.239	11.7 (6.1-15.2)	11.2 (5.8-18.3)	0.469
• Platelets $x10^3/mm^3$	225 (113-308)	290 (110-702)	**< 0.001**	249 (229-386)	295 (110-702)	0.183
• Creatinine, mg/dL	1.0 (0.6-3.0)	0.7 (0.3-1.9)	**< 0.001**	0.7 (0.5-1.0)	0.7 (0.3-1.9)	0.952
• Albumin, g/dL	3.8 (3.1-4.9)	3.9 (2.7-4.6)	0.455	4.0 (3.5-4.49)	3.9 (2.7-4.6)	0.394
• CK-MB, ng/mL	13 (2-300)	27 (0-300)	0.669	16 (1-124)	31 (0-300)	0.548
• cTnT, pg/mL	196 (13-16986)	1086 (67-32430)	**0.025**	1018 (85-6208)	1102 (67-32430)	0.852
Characteristics of myocardial infarction						
Symptoms onset, hours	4 (1-30)	7 (1-60)	0.113	6 (2-34)	7 (1-60)	0.979

NYHA ≥ 2, n (%)	6 (35)	14 (34)	> 0.999	0	14 (41)	0.074
LVEF, %	40 (20-68)	50 (15-70)	**0.032**	52 (38-68)	50 (15-70)	0.214
STEMI, n (%)	17 (100)	30 (73)	**0.023**	6 (85)	24 (70)	0.651
GRACE score, points	118 (77-153)	95 (64-178)	**0.006**	76 (64-90)	100 (67-178)	**0.004**
LAD artery occlusion, n (%)	12 (70)	24 (58)	0.553	3 (42)	21 (61)	0.421
Three-vessel disease, n (%)	3 (17)	11 (26)	0.523	1 (14)	10 (29)	0.651
In-hospital drug therapies						
• Antiplatelets, n (%)	17 (100)	41 (100)	> 0.999	7 (100)	34 (100)	> 0.999
• Heparin, n (%)	17 (100)	41 (100)	> 0.999	7 (100)	34 (100)	> 0.999
• Statins, n (%)	17 (100)	39 (95)	> 0.999	7 (100)	32 (94)	> 0.999
• RAAS inhibitors, n (%)	15 (88)	33 (80)	0.707	7 (100)	26 (76)	0.310
• Thrombolytics, n (%)	8 (47)	23 (56)	0.574	3 (42)	20 (58)	0.678
Days of hospitalization	5 (1-9)	5 (1-44)	0.169	5 (4-11)	5 (1-44)	0.980

Data are expressed as the median (minimum-maximum range) unless otherwise specified. Significant p-values are in bold.

Table 2. Color-flow cytometry assays and cytokine levels among study participants

	Participants grouped by sex		**p**	**Women grouped by menopausal status**		**p**
	Men (n = 17)	**Women (n = 41)**		**Pre (n = 7)**	**Post (n = 34)**	
Flow cytometry assays						

# of $CD4^+$ cells/10,000 PBMC	2801 (485-4332)	3555 (1266-8497)	**0.003**	3555 (2219-4900)	3611 (1266-8497)	0.622
$CD4^+T\text{-}bet^+/CD4^+$ cells, %	28.8 (14.9-49.0)	32.7 (10.0-55.9)	0.184	28.5 (20.2-35.3)	33.9 (10.0-55.9)	0.125
$CD4^+GATA3^+/CD4^+$ cells, %	23.5 (4.3-69.5)	18.4 (6.2-54.3)	0.138	16.8 (9.6-54.3)	19.2 (6.2-38.0)	0.799
$CD4^+ROR\gamma t^+/CD4^+$ cells, %	49.8 (14.1-64.9)	46.6 (20.0-82.5)	0.892	55.0 (24.3-65.1)	42.2 (20.0-82.5)	0.266
Th1/Th2 phenotype ratio	1.1 (0.2-6.9)	1.5 (0.3-7.2)	0.081	1.5 (0.3-3.6)	1.5 (0.5-7.2)	0.773
Serum cytokine levels						
MCP-1/CCL2, pg/mL	–	–		312 (177-500)	234 (45-500)	0.212
Interleukin-6, pg/mL	–	–		20 (7-160)	38 (7-360)	0.544
TNF, pg/mL	–	–		20 (7-126)	7 (7-130)	0.662
Interleukin-1β, pg/mL	–	–		2.3 (2.0-22.6)	5.1 (2.0-31.4)	0.306
Interleukin-10, pg/mL	–	–		3.9 (3.9-22.1)	3.9 (3.9-149.1)	0.409

Data are presented as the median (minimum-maximum values). Significant *p*-value is in bold.