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Peripheral blood immunologic phenotype of population-based breast cancer cases and matched controls

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Reduced breast cancer risk has been seen with the acquired immunodeficiency syndrome (AIDS) and rheumatoid arthritis.[1–3] The CD4+ regulatory subset of T lymphocytes is a major focus of breast cancer pathogenesis,[4, 5] but data on overall differences in peripheral blood mononuclear cell (PBMC) subsets between breast cancer cases and controls are sparse.[6]

We compared PBMC in 72 non-metastatic breast cancer cases (18 in situ, 54 locally invasive; mean age 54; all treated only with surgery) to 91 age-matched general-population controls in Warsaw and Lodz, Poland.[7] Most cases were classified for tumor expression of estrogen receptor (ER), progesterone receptor (PR), HER2/neu (HER2), and epidermal growth factor receptor (EGFR). Each participant provided acid-citrate dextrose anticoagulated whole blood from which PBMC were frozen the same day in dimethyl sulfoxide and maintained at −80C until testing.[8] PBMC immunophenotypes were determined by flow cytometry (Supplementary methods). Coefficients of variation (%CV) in duplicate vials were satisfactory (median 7.8%) except for the uncommon CD8+CD3- subset (36.7%). Case-control differences were tested by Kruskal-Wallis (KW) one-way analysis of variance and mixed models (Statistical Analysis System ver 9.0, Proc Mixed) that adjusted (adj) for 5-year age groups, city, and test date.

Table 1 presents PBMC median values in the cases and controls. Total PBMC (98.5% lymphocytes) were modestly higher in cases than controls (14,132/μL vs 13,701/μL, P_{KW}=0.04). CD19+ B cells were non-significantly higher, and CD3+ T cells were non-significantly lower among cases. Of the CD3+ T cells, cases tended to have lower levels of CD8+ cells (23.2% vs 26.5%, P_{KW}=0.09), as well as non-significantly lower levels of CD4+ cells. CD4/CD8 ratio was non-significantly higher in cases (1.84) than controls (1.77, P_{KW}=0.20).

Among the rarer T-cell populations, cases tended to have modestly higher levels of CD3+CD25+ cells (6.4% vs 5.3%, P_{KW}=0.02), CD4+CD25+ cells (4.6% vs 4.2%, P_{KW}=0.11), and CD4-FoxP3+ cells (0.20% vs 0.15%, P_{KW}=0.05). Unexpectedly, CD4+FoxP3+ cells tended to be lower in cases (0.7% vs 1.1%, P_{KW}=0.10). CD3+CD56+ natural killer (NK) T cells also tended to be lower in cases (5.8% vs 6.8%, P=0.12), in

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The authors declare that they have no competing interests.

contrast to CD3-CD56+ NK cells, which were non-significantly higher in cases. Cases tended to have higher levels of CD80+ lymphocytes (2.1% vs 1.7%, P_{KW}=0.06), as well as non-significantly higher levels of CD80+ monocytes (1.8% vs 1.2%, P_{KW}=0.15).

With multivariate adjustment for age, city, and test date, CD4/CD8 ratio was significantly higher in cases (P_{adj}=0.001). With adjustment, cases and controls had no other significant differences, although cases tended to have more total PBMC (P_{adj}=0.06) and fewer CD8+CD3+ T cells (P_{adj}=0.06).

Cases in all oncogene-defined strata (ER, PR, HER2, and EGFR) had more total PBMC than controls (P_{KW}=0.01 to 0.06, Supplementary Table 1). Similarly, CD3+CD25+ cells were significantly elevated in all oncogene-defined strata of cases (P_{KW}=0.01 to 0.05) except HER2-positive cases (P_{KW}=0.56). CD4/CD8 ratio differed in all strata only after adjustment by mixed modeling; this was noteworthy with stratification for PR (P_{adj}=0.0001). Other associations of nominal statistical significance (not presented) were either inconsistent across strata, or they were not confirmed by adjusted, mixed model analysis.

Our population-based study found no major differences in PBMC levels between the general population of women with node-negative breast cancer and women randomly selected from the same general population. As observed elsewhere,[6] CD8+ T cells tended to be lower in cases than in controls. Because cases had more total PBMC than did controls, a slightly higher deficit in total CD8+ cells among cases may have been hidden.

Our study had several weaknesses, especially our use of frozen whole blood specimens. We used state-of-the-art flow cytometry that yielded highly reproducible results for common PBMC subsets in masked paired vials in the current study and previously,[8] but accurate enumeration of rare cell populations may have been affected by freezing, thawing, and staining. We mitigated technical weaknesses by interspersing and identically handling specimens from cases and controls. Finally, we did not examine CXCR4, signaling of which may trigger apoptosis of neoplastic breast cells and account for the breast cancer deficit in women with AIDS.[9, 10] This should be considered in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Peripheral blood mononuclear cell (PBMC) phenotype levels in 72 breast cancer cases and 91 matched controls.

Marker	Median values* (No.) cases	controls	Kruskal-Wallis P-value	Adjusted P-value†
Total PBMC	14132 (72)	13702 (91)	**0.04**	**0.06**
Lymphocytes (%)				
Total lymphocytes	98.5 (72)	98.5 (91)	0.52	0.73
CD19+ (B cells)	11.4 (69)	11.1 (90)	0.32	0.37
CD3+ (T cells)	73.4 (71)	74.3 (91)	0.18	0.40
CD8+CD3+	23.2 (71)	26.5 (91)	**0.09**	**0.06**
CD4+CD3+	44.8 (71)	46.3 (91)	0.78	0.87
CD4/CD8 (ratio)	1.84 (71)	1.77 (91)	0.20	**0.001**
CD3+CD25+	6.4 (72)	5.3 (91)	**0.02**	0.37
CD4+CD25+	4.6 (72)	4.2 (91)	0.11	0.17
CD4+FoxP3+	0.7 (70)	1.1 (85)	0.10	0.58
CD4−FoxP3+	0.20 (70)	0.15 (85)	**0.05****	0.49**
CD8+CD3−	3.5 (71)	3.4 (91)	0.67	0.99
CD3+CD56+	5.8 (69)	6.8 (90)	0.12	0.69
CD3−CD56+ (NK cells)	12.2 (69)	10.9 (90)	0.30	0.34
CD80+	2.1 (71)	1.7 (91)	**0.06**	0.54
Monocytes (%)				
CD54+	99.0 (69)	98.8 (89)	0.80	0.48
CD80+	1.8 (69)	1.2 (89)	0.15	0.73

*Percent (%), except total PBMC (cells/μL) and CD4/CD8 ratio.

†Adjusted for age group, city, and test date by Poisson or mixed model procedures, as appropriate for the distribution.

**Extremely high outlier value in one subject (a case) excluded.