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Changes in peripheral blood eosinophils may predict colorectal cancer – A retrospective study

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ABSTRACT

Background: Colorectal cancer (CRC) is a leading cause of morbidity and mortality worldwide. Eosinophils are traditionally associated and studied in context of allergic diseases. However, recent data implicate their involvement in mucosal tumors, especially in CRC where they may have an anti-tumorigenic function.
Our objective was to evaluate whether trends in peripheral blood eosinophil numbers are associated with future diagnosis of CRC.

Methods: This retrospective cohort study included adult patients diagnosed with CRC compared to matched controls. We evaluated the linear change in the absolute number of eosinophils (ANE) in peripheral blood over time, described as a correlation coefficient (r). The timeline started 7 years and ended 3 months before diagnosis of CRC.

Results: We included 8334 CRC patient/control pairs. Over the study period, no linear correlation was found between levels of eosinophils and time in either group. In a subset of patients (1350, 8.1%), a positive linear correlation was found between levels of eosinophils and time. CRC was significantly more common in these patients (59% vs. 41%, $p < 0.01$). In a logistic regression, positive r was found to be an independent predictor for CRC (OR 1.31, 95%CI: 1.22–1.41, $p < 0.001$) with high specificity (0.93) but low sensitivity (0.1).

Conclusion: We found higher risk for CRC in patients with a positive linear increase in peripheral eosinophils over time. This may be an indirect clue that eosinophils play a role in the pathogenesis of CRC. Linear changes in ANE may be used in the future to improve screening measures for CRC.

Trial registration: Not relevant.

Keywords: Eosinophils, Cancer, Colorectal, Prognosis, Screening

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INTRODUCTION

Colorectal cancer (CRC) is one of the leading causes of morbidity and mortality in the western world. Although extensively investigated, the reasons for the heterogeneous course and prognosis of CRC are not entirely understood. Over the past few years, it has become increasingly apparent that cells in the tumor microenvironment, and especially immune cells, have a significant role in tumor progression and resistance to therapy.[1]

Eosinophils are bone marrow-driven leukocytes that have been largely studied in the context of allergic diseases and parasitic infections.[2] Nonetheless, eosinophils infiltrate multiple types of tumors, including bladder, gastric, esophageal, head and neck, lung, liver, ovary and uterine cervix.[3] Subsequently, their role in malignancies has gained recent attention.[4,5] Specifically, eosinophils are abundant in tumors of mucosal origin and especially in the tumor microenvironment of patients with CRC.[6] Experimental murine models of CRC display increased eosinophilic infiltration as well, and their presence is associated with direct anti-tumorigenic activities independent of $CD8^+$ lymphocytes.[7] In support of these findings, the presence of eosinophils in human CRC biopsies was correlated with prolonged remission, better prognosis, and reduced CRC-related mortality.[8,9] Accordingly, it has been suggested that tumor-associated tissue eosinophilia should be a positive criterion in disease staging.[10] Collectively, these data suggest that eosinophils may have an anti-tumorigenic function in CRC.

Complete blood count (CBC) is one of the most frequently performed laboratory examinations. Thus, the eosinophil count in peripheral blood is available for most adults. Although eosinophils are primarily found in the tissues, in several diseases such as asthma, peripheral blood eosinophil counts correlate with their presence in affected tissues and even disease severity.[2,11,12] Nonetheless, the correlation between the number of eosinophils in the peripheral blood and CRC pathogenesis has not been established.[13]

In the current study, we evaluated whether linear trends in the number of eosinophils in peripheral blood can be associated with future diagnosis of CRC.

MATERIALS AND METHODS

Data source

Clalit Health Services (CHS) is the largest of the four Health Maintenance Organizations in Israel, serving nearly 5 million patients. It is both an insurer and provider. CHS created a centralized electronic warehouse where primary care, specialist, hospital, pharmacy, and laboratory records, in addition to demographic and socioeconomic information, are stored. For this retrospective cohort study, data of all CHS members were accessed from its integrated data warehouse. The data are comprehensive, as members receive most of their care within CHS. Data were extracted using the Clalit Research Data sharing platform powered by MDClone (https://www. mdclone.com).

Study cohort

All CHS members ages 21 or older, diagnosed with CRC during the study period of January 2011 to December 2018, were included. The diagnosis was identified using the International Classification of Diseases, Ninth Revision (ICD-9). To further confirm the diagnosis, data were compared to the National Cancer Registry, and in all patients diagnosed with CRC, CEA and/or CA19-9 blood levels were checked. Only patients with a confirmed diagnosis were included.

The control group consisted of CHS members ages 21 or older without CRC. They were matched 1:1 to the study group by age, sex and Charlson comorbidity index (CCI) score.

Patients in the study and control groups were included only if they had at least 3 CBC results in the 7 years prior to the diagnosis of CRC and until 3 months prior to the date of diagnosis (index day). Patients with medical conditions including atopy, eosinophilic granulomatosis with polyangiitis, or hypereosinophilic syndrome were excluded. Patients on medications that may alter eosinophil counts were excluded as well. These medications included use of system steroids or anti-parasite during study period. The final cohort included well-matched patient-control pairs, eligible according to the above criteria.

The study evaluated the *linear* relationship between the absolute numbers of eosinophils (ANE)

in peripheral blood and time, described as a Pearson correlation coefficient (r). r was calculated by using Pearson's correlation coefficient formula between the number of days before the index day and ANE for each patient. The timeline started 7 years before diagnosis of CRC and ended 3 months before diagnosis. Positive r describes a linear increase in the ANE over time and vice versa. The closer r is to 1/-1, the more linear the correlation. r values close to 0 indicate no linearity.

Statistical analysis

Demographics and CCI score at baseline were presented as absolute numbers and percentages, when appropriate. General linear model (GLM) repeated measures were employed to examine differences in the average CBC test numbers and in ANE between the two groups (case and matched control). Chi-square test was used to compare proportions for the categorical variables between those with $r > 0.66$ and those with $r < 0.66$ in the entire population and among case group. The continuous variables, dependent variables were compared using the *t*-test.

General linear model repeated measures were conducted to test whether there were significant differences in ANE correlation coefficients between the two study groups. The GLM was conducted as univariate without controlled variables and as multivariate with the following controlled variables: the difference in the number of eosinophilic tests done among each pair (case-control), age and sex.

A binary logistic regression was performed to determine the risk of developing CRC based on the ANE correlation coefficient.

Considering the CRC diagnosis as a gold standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ANE correlation coefficients were evaluated.

Chi-square test was used to compare proportions for CRC when we divided the cohort into 6 subgroups, according to the ANE correlation coefficient. All statistical analyses were performed with SPSS/PC statistical software, version 27.0. Two-sided tests of significance $p < 0.001$ were used in all analyses.

RESULTS

Patient cohort

During the study period, 9232 patients diagnosed with CRC were identified. Of which, 8923 had at least 3 CBC test results in the 7 years preceding the diagnosis of CRC and until 3 months prior to the index date. After matching them with the control group, 8334 CRC patient/control pairs were eligible, according to the study criteria.

Demographics are summarized in Table 1. Sex distribution had a slight female predominance (4395 pairs, 52.7%). Most patients (96.3%) were older than 50 years, and 73.6% had a low-intermediate CCI score. According to the inclusion criteria, patients and controls had at least 3 CBC during the study period. The control group had significantly more CBC tests during the study period than the study group (14.4 ± 16.8 vs. 11.3 ± 7.8, respectively; $p < 0.001$).

Linear increase in ANE over time is a positive predictor for CRC

Eosinophil counts were extracted from the CBC. At baseline, the average number of eosinophils in the study group was 0.21 ± 0.16 $10^3/\mu L$ vs. 0.22 ± 0.18 $10^3/\mu L$ in the control group ($p = 0.05$). The average number of eosinophils at the endpoint in the study group was 0.22 ± 0.17 $10^3/\mu L$ vs. 0.22 ± 0.22 $10 \times 3/\mu L$ in the controls ($p = 0.313$). The change in the baseline and endpoint eosinophils was greater in the study

Characteristic	n pairs (%)
Female sex	4395 (52.7%)
Age groups	
27–48	311 (3.7%)
49–63	1252 (15.0%)
64–78	3355 (40.3%)
79–93	3057 (36.7%)
≥94	359 (4.3%)
CCI score	
0-1 – low	3130 (37.6%)
2-3 – intermediate	2999 (36.0%)
4-19 – high	2205 (26.5%)

Table 1. Demographics and Charlson comorbidity index (CCI) score at baseline of the study pairs

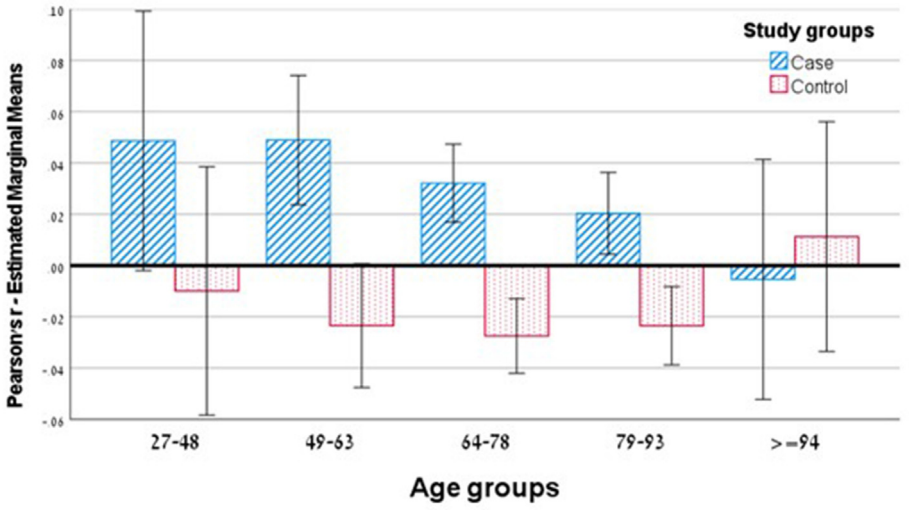

Fig. 1 *Linear* relationship between the ANE and time, described as a Pearson correlation coefficient (r) by age groups

group (0.009 ± 0.15) compared to the control group (0.001 ± 0.20), p = 0.004.

As shown by the Pearson correlation, no linear correlation was found between levels of eosinophils and time during the study period in patients (r = 0.03 ± 0.45) and controls (r = −0.02 ± 0.43). This was consistent after correcting for age and sex (Figs. 1 and 2). Accordingly, the sensitivity of a positive r for CRC was 0.5, with a PPV of 0.53. The specificity was 0.55 with a NPV of 0.52.

When dividing the patients according to r values, we found 1350 (8.1%) who had a very good linear correlation between ANE and time (r > 0.66). Patients with a good linear correlation between ANE and time were younger and with a better CCI score. This finding was constant when compared as a group (Table 2) or when compared within the study population with CRC (Table 3). Nevertheless, the proportion of CRC diagnosis was significantly higher (59% vs. 41%, p < 0.01) in these patients (Table 4). An r > 0.66 was found to be specific for CRC diagnosis (0.93) but with low sensitivity (0.1) (Table 5). In a logistic regression, positive r was found to be an independent predictor of CRC (OR 1.31 95%CI: 1.22–1.41, p < 0.001).

DISCUSSION

Colorectal cancer is a common malignancy with variable course. Although extensively investigated,

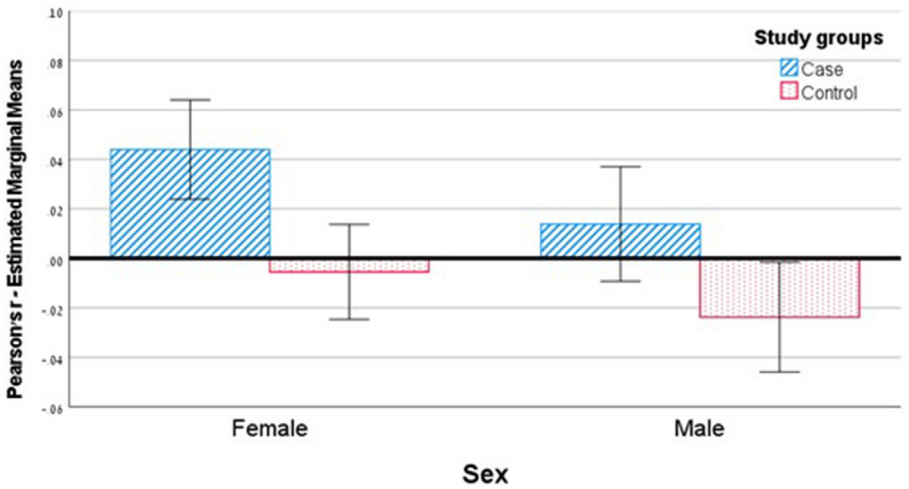

Fig. 2 *Linear* relationship between the ANE and time, described as a Pearson correlation coefficient (r) by sex

Characteristic	r < 0.66 n = 15 318	r > 0.66 n = 1350	p
Female sex n (%)	8100 (52.9%)	690 (51.1%)	.212
Age groups n (%)			
27–48	538 (3.5%)	84 (6.2%)	<.001
49–63	2248 (14.7%)	256 (19.0%)	
64–78	6140 (40.1%)	570 (42.2%)	
79–93	5721 (37.3%)	393 (29.1%)	
≥94	671 (4.4%)	47 (3.5%)	
CCI score n (%)			
0-1 – low	5602 (36.6%)	658 (48.7%)	<.001
2-3 – intermediate	5595 (36.5%)	430 (31.9%)	
4-19 – high	4121 (26.9%)	262 (19.4%)	
Number of CBC test	13.35 ± 13.66	7.61 ± 5.78	<.001
Baseline ANE	0.22 ± 0.17	0.16 ± 0.10	<.001
Endpoint ANE	0.21 ± 0.19	0.33 ± 0.21	<.001
Difference between Baseline and Endpoint ANE	−0.01 ± 0.18	0.18 ± 0.15	<.001

Table 2. Comparison between patients with r > 0.66 and with r < 0.66 in the entire population

the reasons for favorable outcomes or progression are not fully understood.

Eosinophilic infiltration has been shown in different kinds of tumor microenvironments and has been associated with diverse functions.[4] Eosinophils have been recently reported to display anti-tumorigenic activities in two distinct mouse models of CRC.[7] In humans, several studies have shown that the presence of eosinophils in the

Characteristic	r < 0.66 n = 7538	r > 0.66 n = 796	p
Female sex n (%)	3977 (52.8%)	418 (52.5%)	.894
Age groups n (%)			
27–48	261 (3.5%)	50 (6.3%)	<.001
49–63	1097 (14.6%)	155 (19.5%)	
64–78	3011 (39.95)	344 (43.2%)	
79–93	2831 (37.6%)	226 (28.4%)	
≥94	338 (4.5%)	21 (2.6%)	
CCI score n (%)			
0-1 – low	2758 (36.6%)	372 (46.7%)	<.001
2-3 – intermediate	2735 (36.3%)	264 (33.2%)	
4-19 – high	2045 (27.1%)	160 (20.1%)	
Number of CBC test	11.73 ± 7.91	7.55 ± 5.21	<.001
Baseline ANE	0.22 ± 0.16	0.16 ± 0.10	<.001
Endpoint ANE	0.21 ± 0.16	0.34 ± 0.20	<.001
Difference between Baseline and Endpoint ANE	−0.01 ± 0.14	0.18 ± 0.15	<.001

Table 3. Comparison between patients with CRC and r > 0.66 and with r < 0.66

r	All patients	Patients with colon cancer	p-value
≤-0.66	1219	592 (48.6%)	<0.001
−0.66–−0.33	2760	1297 (47.0%)	
−0.33-0	4799	2282 (47.6%)	
0-0.33	3861	1933 (50.1%)	
0.33-0.66	2679	1434 (53.5%)	
>0.66	1350	796 (59.0%)	

Table 4. Risk for CRC diagnosis by Pearsons correlation (r)

r cut-off	Specificity	Sensitivity	PPV	NPV
<0	0.55	0.5	0.53	0.52
<0.33	0.78	0.27	0.55	0.52
<0.66	0.93	0.10	0.59	0.51

Table 5. Specificity and sensitivity for CRC diagnosis by r cut-off values

microenvironment of CRC has beneficial effects on tumor progression and patient prognosis.[9,14,15] Only 1 small cohort investigated peripheral blood counts and presented an inverse association between blood eosinophil count and CRC risk.[13]

In this study, we evaluated the change in ANE in peripheral blood over time, in a large cohort of patients before the diagnosis of CRC, compared to matched healthy controls. The total increase in baseline and endpoint eosinophils was significantly higher in the study group compared to control. Although statistically significant, this difference was too minor to be used in clinical practice. When looking into the pattern of ANE increase over time, we found that when peripheral ANE increased linearly, the risk for diagnosis of CRC was higher.

Eosinophils are bone marrow-derived cells that are usually found in low numbers in the peripheral blood, where they account for as much as 3% of the peripheral white blood cell count. While the majority of studies have focused on assessing eosinophils in the context of allergic inflammation and parasitic infections, it has become increasingly apparent that eosinophils may have homeostatic immunoregulatory activities as well. For example, eosinophils store a plethora of cytokines and chemokines, including IL5, IL4, IFN-γ, IL13, and tumor necrosis factor-α. Furthermore, they have an intricate cross-talk with additional cells that might be present in the inflammatory environment, including T cells, macrophages and dendritic cells. Although peripheral blood eosinophilia was noted in cancer patients more than 100 years ago, their overall activities in the tumor microenvironment and their association with different malignancies remain elusive.

Recent animal studies established an inverse link between eosinophil infiltration and CRC progression. It was speculated that activated eosinophils enhance the effect of cytotoxic T cells, thus, inhibiting tumor growth. The initial trigger for eosinophil recruitment from the bone marrow and the regulatory effects are unknown. Accordingly, it is not clear whether the described eosinophil migration can be tracked. Nevertheless, as opposed to bone marrow or colon pathology, peripheral blood counts, including ANE, are easily accessible. We assumed that changes in ANE in peripheral blood may be associated with their passage from the bone marrow to the evolving tumor.

The study group included patients with definite diagnosis of CRC. The estimated growth rate of

this tumor is slow and time from first replication to clinical signs is estimated to be 5 years. Accordingly, we looked into the linear change in the ANE 7 years before diagnosis and forward. ANE is a robust datum in the adult population, and normally does not change over time or with age.[16]

We found that a positive linear change in ANE years before diagnosis of CRC is an independent predictor of CRC. In patients with a very positive correlation coefficient, the specificity for CRC was found to be high. It is speculated that this linear increment in ANE represents their recruitment to the tumor microenvironment. Furthers studies are needed to verify this correlation.

Along with the pathophysiological findings, ANE changes may be used as an additional screening method for CRC. According to international guidelines, current CRC screening options involve either stool-based tests or invasive imaging.[17] Both types of assessments have substantial limitations: stool examinations have high false-positive rates and low sensitivity. Colonoscopy needs bowel preparation and is invasive. Accordingly, other, noninvasive methods have been proposed. These include fecal, urine, exhaled breath, and blood-based tests.[18,19] All of them promising but still not available for screening large populations. Consequently, more than half of the population eligible for screening avoid it.[20] ANE is one of many biological changes possibly induced by CRC. As such, it cannot be considered solely as a good screening method. However, combining linear changes in ANE with other biological changes, such as decrease in hemoglobin and/or increase in inflammatory markers may increase the sensitivity and specificity of these tests and pose a noninvasive screening alternative.

This study has a few limitations inherent to retrospective studies. Some of the clinical and laboratory information was not available including type of cancer and staging, hemoglobin and inflammatory markers. Other possible remote causes for changes in ANE were not available as well. We did not have histologic data regarding eosinophilic infiltration in CRC of patients and could not prove the correlation between ANE changes over time and eosinophilic infiltration in the tumor or prognosis. Nevertheless, this is the first study to date, demonstrating a potential clinical correlation between linear changes in peripheral ANE levels and CRC in humans.

In conclusion, we found a higher risk for CRC diagnosis in patients with a linear increase in peripheral ANE levels over time. This is an indirect clue, that eosinophils may play a role in the pathogenesis of CRC. Moreover, the changes described in ANE may be used in the future to improve screening measures for CRC.

Abbreviations

ANE, Absolute number of eosinophils; CBC, Complete blood count; CCI, Charlson comorbidity index; CHS, Clalit Health Services; CRC, Colorectal cancer; GLM, General linear model; NPV, Negative predictive value; PPV, Positive predictive value.

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Author contributions

Yossi Rosman: Substantial contributions to the conception or design of the work, drafting the work, final approval of the version to be published and agreement to be accountable for all aspects of the work.
Tzipi Hornik-Lurie: Acquisition, analysis and interpretation of data for the work, drafting the work, final approval of the version to be published and agreement to be accountable for all aspects of the work.
Meir-Shafrir Keren: Acquisition, analysis and interpretation of data for the work, revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work.
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Ariel Munitz: Substantial contributions to the conception or design of the work, revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work.

Confino-Cohen Ronit: Substantial contributions to the conception or design of the work, revising it critically for important intellectual content, final approval of the version to be published and agreement to be accountable for all aspects of the work.

Statement of ethics
The formal name of the ethic Committee is "the ethic committee of the Meir medical center". Approved case number is 0078-19-MMC.

Consent for publication
We consent that the manuscript will be published in the World Allergy Organization Journal.
All data and materials are available upon request.

Submission declaration
The above article is original and has not been submitted for publication elsewhere. Our article has been written read and approved by all authors. All requirements for authorship have been met and we declare no conflicts of interest for each named author.

Declaration of competing interest
The authors have no conflicts of interest to declare.

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