BRIEF REPORT

Elevated eosinophil levels observed in infantile hemangioma patients from Kaifeng, China [version 1; peer review: 2 approved]

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Abstract
Infantile hemangioma (IH) is one of the most common soft-tissue neoplasms of infancy. Although clinical diagnosis for IH is well-established, the haematological parameters associated with IH are not well explored. In this short study, we observed significantly higher eosinophil (EO) numbers in IH patient blood compared to healthy controls. This contributed to the observed higher EO % in the peripheral blood of IH patients and was irrespective of age. This new haematological finding could carry a potential diagnostic/prognostic relevance for IH.

Keywords
Infantile hemangioma, eosinophil, haematology, China

Open Peer Review

Reviewer Status ✓ ✓

	Invited Reviewers	
	1	**2**
version 1 16 Dec 2019	✓ report	✓ report

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2. **Swaminathan Sethu**, Narayana Nethralaya Foundation, Bangalore, India

Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Infantile hemangioma (IH) is a common benign tumour in children that presents as precursor vascular lesions, which either present at birth or develop during the early neonatal period and undergo rapid proliferation[1]. IH is the most common vascular tumour of infancy, occurring in up to 10% of infants[2] and is characterized by high expression of genes involved in vasculogenesis, angiogenesis and tumorigenesis[3]. In the Chinese population, low birth weight, prematurity and maternal progesterone have been associated with IH development[4]. Although clinical diagnosis for IH is well-established, other than the proposed embryonic stem cell origins of IH[5], little is known about the peripheral blood cell repertoire in IH patients, let alone in Chinese patients. This concise study seeks to determine any potential haematological signature(s) that may be present in the peripheral blood of IH diagnosed Chinese patients. In this retrospective study, we report significantly elevated eosinophil numbers in Chinese IH patients.

Methods

Kaifeng Central Hospital (Kaifeng, China) is designated as a health care centre by the Kaifeng city government. Retrospective analysis of Kaifeng Central Hospital patient records was performed for this study and the study protocol was approved by the Kaifeng Central Hospital Ethics Committee, which waived the need for informed consent from patients/guardians for the use of their records. Underlying data are all de-identified demographic variables and blood parameters for each individual patient[6]. Patients' parents/guardians had been made aware that this data could be used for research purposes.

Study subjects included paediatric patients (n = 1631) of all sexes (Male (M) = 460 / Female (F) = 1171) between the ages of 0 to 12 months (3.77 ± 2.98 months, mean ± SD) who were diagnosed with IH from January 2011 to December 2016. Control subjects (n=1602) were healthy children who had blood taken during routine medical check-up visits to the hospital during that same period. As previously seen[7], we observed significantly more female IH patients than males (Chi squared test, $p<0.001$). The inclusion criteria included only infants up to 12 months of age and infants with all variables measured (WBC, RBC, MPV, HGB, PCT, EO%, EO#). The exclusion criteria were subjects with other existing conditions and diseases including eczema, systemic infection, allergy, haematological diseases, immunological diseases and adrenocortical insufficiency and who were not undergoing treatment for IH.

Peripheral blood samples (n = 3233) were assayed for full blood panel count on the Sysmex XN-800i (Sysmex Europe GmbH, Norderstedt, Germany) as per manufacturer's protocol. Blood variables measured included white blood cell (WBC) counts, red blood cell (RBC) counts, mean platelet volume (MPV), haemoglobin (HGB) levels, procalcitonin (PCT) levels and eosinophils (EO) percentage/counts.

Due to strong non-normality of some variables the non-parametric Mann-Whitney Test was used in the analysis of continuous variables. Chi-Square test of independence was used for categorical data. All statistical analysis was done on IBM SPSS Statistics 22.0 (SPSS Institute, Chicago, IL, USA). Before analysis, all variables were reviewed for accuracy of data entry and missing values. Due to the large sample size involved, statistical analysis is focused primarily on frequencies and percentages.

Results

We analysed blood parameters between IH patients and healthy controls (Table 1). Notably, we observed a high elevation of EO numbers in IH patients compared to healthy subjects. Compared to the healthy control ($0.19\pm0.24 \times 10^9$/ µL), there is an almost significantly (Chi-Square test of independence, $p<0.001$) two-fold higher EO count in IH patients ($0.4\pm0.37 \times 10^9$/ µL). This contributed to the observed higher EO % in the peripheral blood of IH patients.

This observation was irrespective of age as significantly higher EO numbers (Mann-Whitney test, $p<0.001$) were observed only between IH patient and healthy control cohort for each age-matched group, not between each age group (Table 2). Other measured blood parameters were comparable between IH patients and healthy controls (Table 1).

Discussion

Elevated EOs are classically associated with the presence of inflammation in patients with conditions such as asthma, allergy and parasitic infections. Our exclusion criteria in this study discounted any possibility of this on our observations. Previous haematological analyses of blood collected from 34 IH patients

Table 1. Haematological profile in healthy and infantile hemangioma subjects.

	IH (n=1631)	Control (n=1602)	P-value
Age (in months) Mean (SD)	3.77 (2.98)	3.44 (2.67)	0.016[1]
Median (IQR)	3 (5)	2 (4)	
0–3 months N (%)	958 (50.0)	958 (50.0)	
4–6 months N (%)	364 (50.4)	358 (49.6)	
6–12 months N (%)	309 (51.9)	286 (48.1)	
Gender Male N (%)	460 (38.5)	736 (61.5)	<0.001[2]
Female N (%)	1171 (57.5)	866 (42.5)	
WBC (10^9/µL)	10.10 (3.21)	10.39 (4.38)	
RBC (10^6/µL)	4.33 (0.69)	4.79 (0.51)	
MPV (fL)	9.74 (0.81)	9.47 (0.81)	
HGB (g/L)	113.59 (16.90)	122.74 (14.78)	
PCT (%)	0.05 (0.03)	0.04 (0.02)	
EO %	3.96 (2.46)	1.91 (2.11)	<0.001[1]
EO # (10^9/ µL)	0.40 (0.37)	0.19 (0.24)	<0.001[1]

Control – Healthy subjects, IH-Infantile hemangioma patients, WBC- white blood cells, RBC- Red blood cells, MPV – Mean platelet volume, HGB-Hemoglobin, PCT-Procalcitonin, EO-Eosinophils

[1] Mann-Whitney Test

[2] Chi-Square Test of Independence

Table 2. Comparison of the levels and percentage population of eosinophils among different age groups between healthy and infantile hemangioma subjects.

	IH mean (SD)	Control mean (SD)	P-value
Aged 0–3 months EO %	3.90 (2.47)	1.88 (2.18)	<0.001[1]
EO #	0.42 (0.36)	0.19 (0.25)	<0.001[1]
Aged 4–6 months EO %	4.28 (2.64)	1.84 (1.84)	<0.001[1]
EO #	0.42 (0.45)	0.19 (0.22)	<0.001[1]
Aged 7–12 months EO %	3.77 (2.15)	2.10 (2.17)	<0.001[1]
EO #	0.34 (0.23)	0.20 (0.22)	<0.001[1]

[1] Mann-Whitney Test

in an Italian study revealed slightly elevated EO %[8], but IH blood parameters were not compared to that in healthy subjects. Mean EO reference numbers in the general Chinese population are between 0.1 – 0.2 × 10^{9}[9], in concordance with healthy EO levels we observe.

One major limitation in this study is the inability to compartmentalize IH patients into different clinical phases (i.e. proliferating phase, early regressing (involuting) phase, and advanced regressing (involuted) phase) as this information was not made available to us during retrospective data collection. Future work will focus on determining whether EO numbers increase progressively throughout the different IH clinical phases.

Propranolol, a beta-blocking agent, has been used as the first-line therapy for the management of IH since 2008[10]. However, propranolol use for managing IH in China only came about after findings from a prospective 2011 trial[11]. Given that propranolol has been shown to prevent the release of EO-activating cytokines[12], propranolol would work favourably in IH patients to reduce the abnormally high EO numbers seen in our patients. In this present study, we show for the first time a significant elevation in EO numbers in IH paediatric patients and this could potentially carry a diagnostic/prognostic relevance in Chinese children. IH is commonly diagnosed clinically based on natural history of the lesion. Currently, the most important marker to accurately diagnose IH is glucose transporter 1 (GLUT1)[13], though this marker is present despite the proliferative activity of the IH lesion[14]. The use immune cytokines as a potential biomarker for IH progression was recently proposed[8,15] and some of those cytokines (e.g. interleukin -8) could directly impact EO proliferation. Standard haematological (e.g. abnormal EO numbers) and unique cytokine signatures could potentially serve as a diagnostic/prognostic marker for IH progression.

Data availability

Open Science Framework: Elevated eosinophil levels observed in infantile hemangioma patients from Kaifeng, China, https://doi.org/10.17605/OSF.IO/P8XR3[6].

This project contains the following underlying data:

- raw data_Li *et al.*xls (Raw haematological data)

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Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 27 January 2020

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✔ **Swaminathan Sethu**
GROW Research Laboratory, Narayana Nethralaya Foundation, Bangalore, Karnataka, India

The following are minor suggestions that may improve the clarity and interpretation of the data

1. The title can be “Elevated peripheral blood eosinophil levels in infantile hemangioma patients” or “Elevated peripheral blood eosinophil levels in Chinese patients with infantile hemangioma”.

2. The authors have pointed out that IH is significantly higher in females compared to males. It would be useful to analyse and represent the results in table 1 and 2 based on gender. In other words, in addition to the current statistical analysis, it would be interesting to know whether the eosinophil (EO) levels were significantly different between controls and IH in males and females subjects separately. The authors can also expand the Table 1 parameters based on gender as well.

3. Further, it will be useful to know the normal range for EO in pediatric population. The authors have mentioned the range for Chinese adult population. The authors can calculate the proportion (%) of subjects with IH above the normal range (if available) or the proportion (%) of subjects with IH above the median level in the control group. The authors can also attempt AUC analysis, if possible to improve the clinical relevance of EO levels in the diagnosis of IH.

4. It would be useful to know whether the authors had access to the proportions of other leukocyte subsets other than EO. This would be relevant and the authors can consider addressing this in the discussion.

5. It is unclear how the % of Procalcitonin (PCT) was computed and whether it is possible to include the concentration range for the same. Further, it was not stated as to why PCT was included in the inclusion criteria and how it is relevant to IH or EO levels in the context of IH.

6. Could power calculation be done to show the robustness of the finding with relevance to the sample size?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology; Immunophenotyping; Immune cell subset variations in health and disease

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 02 January 2020

https://doi.org/10.5256/f1000research.23816.r57886

Mark I.R. Petalcorin
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In this concise article, Li and co-authors reported that the infantile hemangioma (IH) patients showed significantly higher eosinophil levels more than the healthy control subjects suggesting a potential diagnostic relevance. Although promising, the article requires more in-depth analysis especially in addressing the causation factors that result in the increase of eosinophil levels.

I have some concerns on the data analysis. Firstly, the mean values of percent eosinophils observed in IH patients of 3.96 and in healthy subjects of 1.91, as shown in Table 1, are both still within the normal clinical range of 0-6% EO (Medscape). Thus, the elevated eosinophil levels in IH

patients might be interpreted as physiologically irrelevant by clinicians as the values are still within the normal range.

Secondly, whilst the difference of %EO values between IH and healthy subjects is statistically significant, the raw data show that only 15% of the total IH patients and about 5% of the healthy subjects have high %EO values above the normal range of 6%. It would be useful if the authors will include this in their analysis considering the clinical implications. In addition, the authors should also specify what is the normal range of %EO used in China as this can vary in different clinical laboratories.

Lastly, I think that there are other factors contributing to the observed increase of eosinophil levels that might be present but not measured in this study such as drug treatment given to IH patients, which could be the underlying cause of the increase but not taken into account. This is the limitation of a retrospective study such as this, in which the authors have no control on how the data were designed and collected, and whether the patients received drug treatment or not. The claim of a potential diagnostic usefulness for this study is an overestimation as the causation is not well-established but only through the association. But this is a good pilot study to test the hypothesis that can be further explored.

As a minor comment, it would be useful to include the units used for all the parameters mentioned in the raw haematological data.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: clinical chemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.