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Elevated blood eosinophils in early infancy are predictive for atopic dermatitis in children with risk for atopy

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Abstract

Background: Accessible markers to predict the development of atopic diseases are highly desirable but yet matter of debate.

Objective: We investigated the role of blood eosinophils at 4 weeks and 7 months of life and their association with developing atopic dermatitis (AD) in a birth cohort of children with atopic heredity.

Methods: Infant blood samples for eosinophil counts were taken from 559 infants at 4 weeks and from 467 infants at 7 month of life with at least one atopic parent. Elevation of blood eosinophils was defined as $\geq 5\%$ of total leukocytes and the association for the occurrence of AD was assessed by entering 2x2 tables and the odds ratios were estimated followed by hypothesis testing against the alternate working hypothesis: odds ratio $\neq 1$. Survival analysis was carried out estimating the Kaplan-Meier product limit estimator from the life-time table of AD score and time to AD manifestation stratified by the eosinophil binary score.

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Results: Elevated blood eosinophils observed at 4 weeks were significantly associated with the occurrence of AD in the whole cohort at the age of 7 months ($p=0.007$), one year ($p=0.004$), two years ($p=0.007$) and three years ($p=0.006$) of life. AD occurred app. 12 weeks earlier in infants with elevated blood eosinophils at 4 weeks of life. Blood eosinophil counts $\geq 5\%$ at 7 months of life failed to show significance for AD; for eosinophils at 4.5% a significant association at 7 months ($p=0.005$), and one year of life ($p=0.039$), two years ($p=0.033$) and three years ($p=0.034$) was observed.

Conclusion: Elevated blood eosinophils at age 4 weeks have a predictive value for the onset of atopic dermatitis in infancy and early childhood in children with high risk for atopy. Early eosinophil counts may therefore be helpful for counseling parents and to identify individuals for interventional trials aiming at allergy prevention.

Key words: allergy atopic dermatitis atopy prediction blood eosinophils infancy

Abbreviations: AD atopic dermatitis, EE elevated blood eosinophils, DH double parental heredity, SH single parental heredity, MH maternal heredity, PH paternal heredity, TSG total study group, PPV positive predictive value Correspondence: Dr. med. Siri Rossberg Department for Pediatric Pneumology and Immunology Charité University Hospital Berlin Augustenburger Platz 1 13355 Berlin siri.rossberg@charite.de

Introduction:

The development of allergic diseases is determined genetically¹⁻³, epigenetically⁴ and environmentally^{5,6} and starts as early as in the fetus. A pivotal prerequisite for successful primary and secondary allergy prevention is thus to identify individuals at risk for atopy very early in life. Numerous attempts have been made to identify suitable predictive markers to assess individual risk and better understand the course of atopic diseases.⁷⁻¹⁷ Estimating different risk categories such as single or double heredity for atopy in combination with biological markers may help to narrow down optimum candidates for interventions. Eosinophils are known to be involved in allergic and infectious immune responses.¹⁸⁻²² They derive from bone marrow and migrate into the tissue via the blood. In hypereosinophilic conditions they accumulate in the blood and in tissues or both.²⁰ The regulative potential of eosinophils in both innate and adaptive immune responses has been realized over the past decade and became the target for therapeutic strategies in allergic diseases as well as

malignancies.¹⁹ Eosinophils are frequently increased in childhood asthma and AD.^{12,23,24} AD patients with eosinophilia (>500 cells/ μ l) showed high symptom scores and long duration of the disease.¹² Numerous studies showed an association of blood eosinophilia in wheezing episodes to predict recurrent wheeze or asthma in children²⁵⁻²⁸, one study specifically in wheezing-free episodes.²⁹ This indicates that eosinophils may link viral or bacterial infections and atopic predisposition in the development of childhood asthma.³⁰ Blood eosinophilia (defined as >4% of total leukocytes) is one criteria for the Asthma Predictive Index assessing the risk to develop asthma later.³¹ Infant blood eosinophilia at 3 days and 3 months of life predict diverse atopic diseases.^{7-11,32} Contributing to the discourse, we studied blood eosinophils at 4 weeks and 7 months of life and their association with developing AD until 3 years of life in a birth cohort of children with high risk for atopy enrolled in a randomized controlled trial for prevention of AD.³³

Methods:

Study population

The study was part of a randomized placebo-controlled trial on primary prevention of AD (registration no. ISRCTN60475069). 606 healthy infants with at least one atopic parent (defined to have self reported asthma and/or allergic rhinitis and/or food allergy and/or AD) received an oral treatment with either a bacterial lysate (n=303; Pro-Symbioflor®: heat killed *Escherichia coli* DSMZ 17252 and *Enterococcus faecalis* DSMZ 16440) or placebo (n=303) applied three times daily from 5 weeks until 7 months of life. Participants were clinically examined for the development of AD during the intervention period and until the age of 3 years.³³ The study was approved by the Charité Ethics Committee in 2002.

Blood eosinophil counts and total IgE

Blood samples to assess blood eosinophil counts and total IgE were collected and assessed at 4 weeks of life prior to treatment start and at 7 months of age at the end of the intervention. Blood eosinophils undergo circadian variation, so blood samples were taken pre-midday.⁷ Recruitment took place throughout all seasons from 2002 to 2007. Differential blood cell counts were assessed automatically by flow cytometry (Sysmex Analyzer XE-2100 and Sysmex Analyzer XE-5000, Kobe, Japan) and manually. 100 leucocytes were enumerated after combined staining with May-Grünwald (eosin-methylenblue) und Giemsa (azur-eosin-methylenblue). 223 from 672 differential blood samples had to be converted from cells/ μ l into % of total leucocytes. Both data sets of converted and original blood

eosinophils in % showed equal distribution when assessed by scatter plot and rank sum test (data not shown).

Total IgE serum levels were quantified by fluorescent enzyme immunoassay (ImmunoCAP FEIA, Phadia, Freiburg, Germany) and expressed in kU/l. Total IgE was defined as above normal range at 4 weeks of life >1.5 kU/l and at 7 months >25 kU/l according to general recommendation.³⁰

Diagnosis of AD

AD was clinically assessed by using a score according to Hanifin and Rajka³⁴ adapted for infants at 3, 5, 7 months, one year, two and three years of life by pediatricians. The diagnosis AD was made when participants showed itchy eczematous skin lesions at flexural/extensor sites, the face, or both and a typical dryness of the skin exceeding 6 weeks. The primary outcome prevalence of AD at 7 months of life showed no difference between the intervention groups in the total study group (TSG). Due to diverse genetic risk children with one atopic parent were subgrouped for single heredity (SH), children with two atopic parents for double heredity (DH). In the active group (n=303) 148 had SH vs DH, in the placebo group had SH vs DH. AD prevalence was significantly reduced in the subgroup of infants with SH for atopy (10% active group vs. 19% placebo group). In the TSG 156 children developed AD, 107 (17,7%) had mild AD (Scorad <20), 46 (7,6%) moderate AD (Scorad 20<=40) and 3 (5%) cases of severe AD were observed.

Statistical analysis

The descriptive parameters for blood eosinophils were assessed: the arithmetic mean, standard deviation, min, 1, 5, 25, 50, 75, 95 and 99 % percentiles and max values. Since blood eosinophils were not normally or log-normally distributed non-parametric Mann-Whitney rank-sum U test was used to compare blood eosinophils between children with and without AD and between heredity subgroups. Blood eosinophil counts were dichotomized into results below 5% (<5%) and greater than and equal 5% of total leukocytes (≥5%). We defined this as elevated blood eosinophils (EE) according to general recommendations and to provide comparability.^{35,36} Sensitivity and Specificity of blood eosinophils testing for AD were validated by receiver operating characteristic (ROC) analysis (data not shown).

The association of EE measured at 4 weeks and diagnosis of AD at 7 months, one year, two years and three years of life and the blood eosinophil binary score were quantified by bulk odds ratios. The respective 95 % confidence limits were set by hypothesis testing on null hypothesis: odds ratio = 1 against alternate hypothesis: odds ratio $\neq$ 1.

Statistical significance was set at p-value <0.05 .

Survival analysis was carried out estimating the Kaplan-Meier product limit estimator as the eczema free survival time until AD manifestation stratified by the blood eosinophil binary score. The average survival times for the blood eosinophil score were estimated from the Kaplan-Meier survivor functions and confidence limits were estimated by using the Greenwood formula. Log-rank test was used to compare the survival distributions concerning onset of AD in children with and without EE.

Chi Square test for p values was used for the assessment of the association of total IgE >1.5 kU/l at 4 weeks and 25 kU/l at 7 months of life and the diagnosis of AD.

Furthermore, we calculated the positive and negative predictive values, sensitivity, and specificity by cross table for EE at 4 weeks of life, total IgE >1.5 kU/l at 4 weeks of life, SH, SH active treatment, SH placebo, double heredity (DH) and last two combined with EE to assess the predictive potential as in previous studies. Additionally to broaden comparability likelihood ratios (LR+) were calculated.³⁷

Results:

Representing infant blood eosinophil counts in a cohort with risk for allergy we found in the TSG eosinophil levels in peripheral blood to range from 0.9% to 15.1% of total leukocytes at 4 weeks of life, the 50th percentile was at 4%, declining until 7 months of life (Tab1). There was no difference in the distribution of eosinophil counts at the time points 4 weeks and 7 month of life concerning active or placebo treatment for children with single or double heredity (data not shown). Therefore, we examined blood eosinophil counts in all patient groups independently from treatment.

No differences were observed concerning distribution of eosinophils in the TSG and the subgroups of DH and SH (Suppl.Fig 1). Interestingly, no differences were seen in the blood eosinophil counts for male and female participants (data not shown).

Elevated blood eosinophils observed at 4 weeks of life were significantly associated with the occurrence of AD in the TSG at the ages of 7 months, one year, two years and three years of life. (Fig1,Tab2) We found EE reaching a positive predictive value (PPV) of 23.9%, a sensitivity of 52.3% and a specificity of 63.3% in the TSG. Equally predictive was the constellation for DH of 23.3% in this cohort. The best PPV was achieved for the combination of DH and EE with 32.7%. SH alone was not predictive. In the subgroups of SH active vs. placebo PPVs of 7% vs. 18% were observed. (Tab3)

Children with DH suffering from AD at 7 months showed significantly higher counts of blood eosinophils at 4 weeks of life than healthy individuals with DH (Fig2). The subgroups SH, maternal heredity (MH), paternal heredity (PH) showed no such significant differences. There was no association when EE were correlated with specific parental atopic diseases: eczema mother ($p=0.28$), eczema father ($p=0.93$), rhinitis mother ($p=0.32$), rhinitis father ($p=0.79$), asthma mother ($p=0.62$), asthma father ($p=0.76$) (data not shown).

Blood eosinophil counts $\geq 5\%$ measured at 7 months of life just failed to show significance for developing AD ($p=0.06$) at the same age, and were not associated with AD at one year ($p=0.17$), two years ($p=0.14$) and three years of life ($p=0.14$) (data not shown). ROC analysis for sensitivity and specificity assessment showed elevated eosinophils at 4.5% to be significant for the development of AD: 7 months (OR=1.99, 95% CI 1.22–3.25, $p=0.005$), one year (OR=1.65, 95% CI 1.02–2.65, $p=0.039$), two years (OR=1.63, 95% CI 1.04–2.55, $p=0.033$) and three years (OR=1.63, 95% CI 1.04–2.55, $p=0.034$).

Survival analysis revealed a significantly earlier appearance of AD of about 12 weeks in subjects with EE analyzed at 4 weeks of life (log rank test: $p=0.036$) (Fig3). There was no such result for EE at 7 months of life (data not shown). The onset of mild (Scorad Score < 20) and moderate AD (Scorad Score 20–40) was earlier in children with EE. Numbers for severe AD were too small for analysis. In children with EE and moderate AD average time until diagnosis was at 143 days in the placebo and 326 days in the active group (ns).

In the TSG we did not observe an association of total IgE >1.5 kU/l at 4 weeks of life and the occurrence of AD at 7 months ($p=0.75$; OR 0.90), one year ($p=0.95$; OR 0.85), two years ($p=0.81$; OR 0.45) and three years ($p=0.85$; OR 0.57) of life. No difference was

observed for the subgroups SH and DH concerning amount of total IgE at 4 weeks of life in healthy individuals and with AD (data not shown). When total IgE was above 25 kU/l at 7 month of life, a significant association for the presence of AD was seen at 7 months (p=0.002; OR 2.65), one year (p=0.005; OR 2.42), two years (Chi-Square Test: p=0.001; OR 2.65) and three years (p=0.001; OR 2.72) of life (data not shown). We did not find total IgE above normal range at 4 weeks of life to predict AD (PPV 17.2 %, sensitivity of only 16.9% and a specificity of 81.7% (Tab 3). Elevated blood eosinophils at 4 weeks of life were not associated with total IgE >1.5 kU/l at the same time point (p=0.34; OR 1.24), but with total IgE >25 kU/l at 7 month of life (p=0.001; OR 2.82). Elevated blood eosinophils at 7 month of life were associated with total IgE >25 kU/l at the same time point (p=0.001; OR 2.85)(Data not shown).

Discussion

In a birth cohort of children with risk for atopy we found that elevated blood eosinophils measured at age 4 weeks were highly predictive for the occurrence of AD at 7 months, 1 year, 2 years and 3 years of life. Furthermore AD is not only predicted, according to survival analysis AD occurred app. 12 weeks earlier in infants with EE. This supports previous observations that EE reflect a present atopic status in the individual.⁷ The statistical significant PPV in the TSG was found in children with both parents being atopic (DH), not for SH. The risk and prevalence of AD is usually lower when only one parent is atopic, but for the SH subgroup the intervention effect has to be considered. Infants with SH treated with the bacterial lysate had less AD.³³ We hypothesize that the intervention had an effect on lower prevalence and onset of AD (survival analysis) thus EE with SH (active + placebo) had a lower PPV. EE and the development of AD in the subgroups of single heredity active vs. placebo showed indeed PPVs of 7% vs. 18%.

As limitation to this study due to recruitment during all seasons it has to be considered that the pollen season has impact on levels of blood eosinophils in the neonatal period.¹¹ As a confounding factor higher blood eosinophils are observed in newborns during parietaria pollen season, when their mothers are allergic.¹⁶ Borres found blood eosinophilia at 3 months of age linked to early clinical signs of allergic disease (recurrent wheezing defined as asthma, allergic rhinoconjunctivitis, allergen induced urticaria, AD) in atopic individuals until 6 years of life.^{7,32} Elevated blood eosinophils assessed from wheezing infants in an episode of convalescence predicted asthma at 2 to 3 years, 3 to 4 years and 8 to 10 years of life, but

not until adulthood.²⁹ In an ethnically mixed South African cohort with diverse genetic and environmental background cord blood eosinophils were not predictive for the development of atopic disease (asthma, AD, food allergy, allergen induced urticaria) until one year of life.³⁸ In a Japanese cohort study cord blood eosinophilia was significantly associated with infantile eczema at one month of life; latter was associated with AD and recurrent wheezing until two years of life.³⁹ Calbi consistently reported that blood eosinophilia at the third day of life was predictive for atopy (AD, recurrent wheezing, allergen induced urticaria) until 3 years of life in 41 to 54% in a mixed atopic/non-atopic as well as in an atopic cohort.^{11,8} In a larger cohort of 725 infants (553 with and 172 without family history of atopy) the PPV of blood eosinophils $>500/\text{mm}^3$ was found at 47%, total IgE at 12.5%, single parental atopy at 25% and double parental atopy at 40%.⁸ This high predictive value is not present in our cohort, but we found the combination of DH and EE with a PPV of 32.7 % and the LR of 1.6 to have more PPV than the marker cord blood IgE ≥ 0.3 kU/l in combination with DH of 26% as published previously.¹⁴ Controversial results for the PPV of cord blood IgE have been discussed.^{40,41} In a Northern American cohort of 670 participants with a follow-up until adulthood cord blood IgE was not a predictor for allergic diseases showing only a modestly correlation with total IgE and slightly with allergic sensitization.⁴⁰ Increased total IgE levels at 4 weeks of life did not serve as a predictive marker for the development of later AD in this cohort. In general in children younger than 3 years total IgE levels present with a large variation and poor correlation to a specific phenotype or the longitudinal course of an allergic disease.^{16,42}

In this cohort of 50% with single and 50% of the children with double parental atopy, we observed a decline in median blood eosinophils at 4 weeks from 4% to 3% at 7 months of life, independently for maternal or paternal atopy. The decline of blood eosinophils seems a natural phenomenon during childhood and furthermore towards adulthood.^{7,23,32,43} The time window for the prediction of AD by blood eosinophils seems limited. We found no association for EE $\geq 5\%$ at 7 months and occurrence of AD at one year, two years, and three years anymore. Considering the natural decline in blood eosinophils a still significant association was observed for EE at 4.5% of total leucocytes at 7 months and diagnosis of AD for the very same time and clearly with less significant potential for the following than blood eosinophils at 4 weeks. As mentioned, two other cohort studies proved significant association for blood eosinophilia and the development of atopic diseases is known for the first days of life^{8,11} and at 3 months³². Taking also into account the time of onset of AD the assessment for a prediction seems plausible within the first three months of life not later.

Possibly future investigations in cohort studies might also recognize blood eosinophil activation like the expression of the CD69 surface marker and cytokine patterns to better understand and divide pathologic from non-pathologic activation and determine risk categories.²⁴

Using biological markers as a screening method in infants to assess the individual atopic risk is a controversial task, starting with the ethical dilemma of blood sampling. A screening for an increased risk to develop AD may be justifiable if more trials confirm the preventive effect of preemptive skincare in infants.^{44,45}

Conclusion:

Elevated blood eosinophils at age 4 weeks of life have predictive value for the onset of AD in infancy and early childhood in a high risk birth cohort. The assessment seems only to be useful early in infancy, at least within the first three months of life.^{7,8,32} Total IgE had no predictive value in early infancy in this study.

Early eosinophil counts may be helpful for counseling the parents to enhance primary and secondary prevention of AD but furthermore may help to identify individuals for interventional trials aiming at primary prevention.

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Parameter	Blood eosinophils [%]				
	4 weeks of life				
	DH	SH	MH	PH	TSG
N	283	276	150	126	559
Maximum	27.0	24.6	24.6	21.0	27.0
Mean	4.9	4.8	5.0	4.6	4.9
Minimum	0.9	0.9	0.9	1.0	0.9
Standard deviation	3.2	3.0	3.3	2.7	3.1
Coefficient of variation, CV	0.648	0.638	0.673	0.583	0.642
Percentile 99	14.9	16.7	17.4	14.2	15.1
Percentile 95	11.3	10.0	11.0	9.0	10.3
Percentile 75	6.4	6.0	6.0	6.0	6.0
Percentile 50	4.0	4.2	4.7	4.0	4.0
Percentile 25	2.9	2.9	2.8	3.0	2.9
Percentile 5	1.0	1.0	1.0	1.0	1.0
Percentile 1	0.9	1.0	1.0	1.0	0.9
Mann-Whitney U-test between subgroups	p=0.7960		p=0.5104		
Parameter	Blood eosinophils [%]				
	7 month of life				
	DH	SH	MH	PH	TSG
N	235	232	127	105	467
Maximum	15.8	61.0	61.0	15.7	61.0
Mean	3.8	3.9	4.0	3.7	3.9
Minimum	0.2	0.0	0.0	0.8	0.0
Standard Deviation	2.6	4.6	5.7	2.8	3.7
CV	0.671	1.189	1.433	0.741	0.971
Percentile 99	13.3	15.7	26.3	14.8	15.6
Percentile 95	9.0	9.4	9.0	10.2	9.0
Percentile 75	5.0	4.4	4.3	4.5	4.6
Percentile 50	3.1	3.0	2.9	3.0	3.0
Percentile 25	2.0	2.0	1.9	2.0	2.0
Percentile 5	1.0	1.0	1.0	1.0	1.0
Percentile 1	0.6	0.9	0.7	0.9	0.8
Mann-Whitney U-test between subgroups	p=0.1993		p=0.5798		

Tab 1 Distribution of blood eosinophils [%] at 4 weeks and 7 months of life considering heredity subgroups double heredity (DH), single heredity (SH), maternal heredity (MH), paternal heredity (PH) and in the total study group (TSG)

Age	Blood eosinophil count at 4 weeks			
		AD (%)	Healthy (%)	Total
7 months	<5 %	42 (14.3)	252 (85.7)	294
	≥5 %	46 (24.0)	146 (76.0)	192
	Total	88 (18.1)	398 (81.9)	486
	OR 1.89 (95 % CI 1.19-3.01) p=0.007			
1 year	<5 %	49 (16.9)	241 (83.1)	290
	≥5 %	53 (28.2)	135 (71.8)	188
	Total	102 (21.3)	376 (78.7)	478
	OR 1.93 (95 % CI 1.24-3.00) p=0.004			
2 years	<5 %	64 (22.5)	221 (77.5)	285
	≥5 %	62 (33.9)	121 (66.1)	183
	Total	126 (26.9)	342 (73.1)	468
	OR 1.77 (95 % CI 1.17-2.68) p=0.007			
3 years	<5 %	65 (24.1)	205 (75.9)	270
	≥5 %	65 (36.1)	115 (63.9)	180
	Total	130 (28.9)	320 (71.1)	450
	OR 1.78 (95 % CI 1.18-2.69) p=0.006			

Tab 2 Association of normal and elevated blood eosinophil counts at 4 weeks of life and AD prevalence at 7 months, 1 year, 2 years, 3 years of life

	Sensitivity % (95% -CI)	Specificity % (95% -CI)	Positive Predictive Value % (95% -CI)	Likelihood Ratio LR+ (95% -CI)
Eosinophils ≥ 5%	52.3 (41.3-63.0)	63.3 (41.4-63.0)	23.9 (18.1-30.6)	1.4 (1.1-1.8)
Total IgE >1.5kU/l	16.9 (09.8-26.3)	81.7 (77.5-85.4)	17.2 (09.9-26.8)	0.9 (0.6-1.5)
SH	33.7 (24.3-44.1)	48.0 (43.2-52.9)	12.5 (08.7-17.2)	0.6 (0.5-0.9)
SH active	22.5 (10.8-38.5)	44.1 (37.4-51.1)	07.0 (3.3-12.9)	0.4 (0.2-0.7)
SH placebo	41.8 (28.7-55.9)	51.8 (45.0-58.6)	18.0 (11.7-25.7)	0.9 (0.7-1.9)
DPH	66.3 (55.9-75.7)	52.0 (47.1-56.8)	23.3 (18.4-28.8)	1.4 (1.2-1.6)
SH and Eosinophils ≥5%	43.3 (25.5-62.6)	61.4 (54.4-68.0)	14.0 (07.7-22.7)	1.1 (0.7-1.7)
DH and Eosinophils ≥5%	56.1 (42.4-69.3)	65.4 (58.2-72.2)	32.7 (23.5-42.9)	1.6 (1.2-2.2)

Tab 3 Sensitivity, specificity, positive predictive value and likelihood ratio of blood eosinophil counts at 4 weeks, total serum IgE levels at 4 weeks, heredity and in combination for the development of AD. DH double heredity, SH single heredity.

Blood Eosinophils in % of total leucocytes at 4 weeks

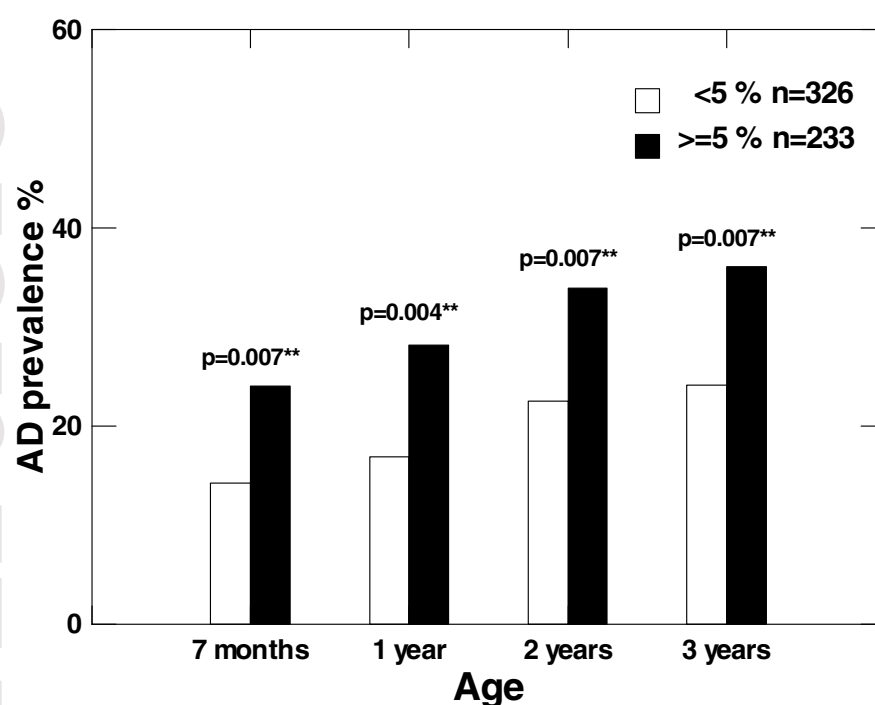


Fig 1 Diagnosis of AD at 7 months, one year, two years, three years of life in association with normal and elevated blood eosinophil counts measured at age 4 weeks by hypothesis testing in odds ratio < 1

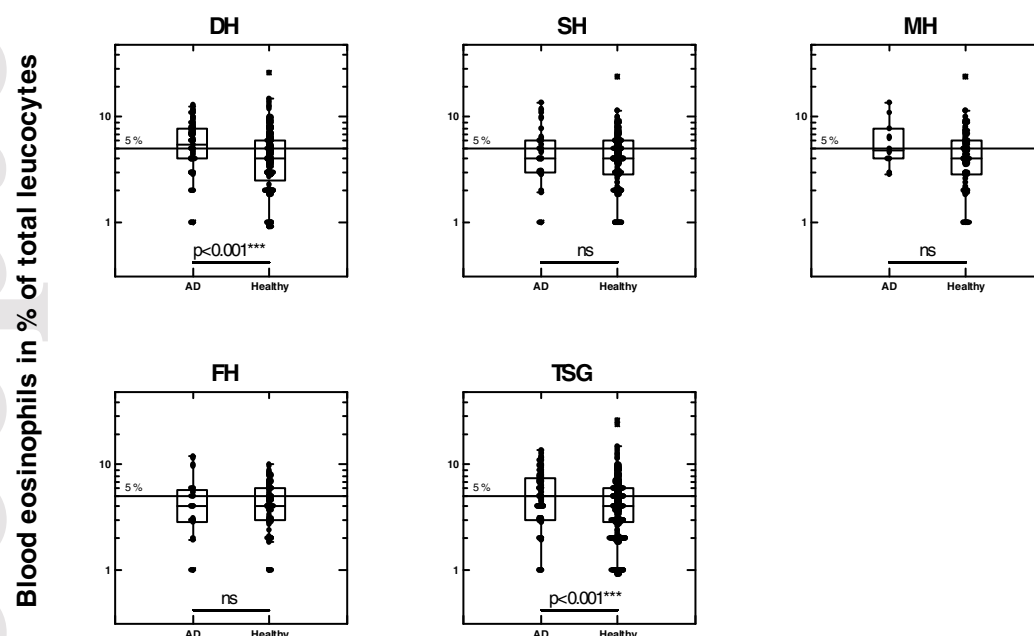


Fig 2 Distribution of blood eosinophil counts in % measured at age 4 weeks in children with and without AD at 7 months of life with DH, SH, MH, PH and in the TSG (Mann-Whitney rank-sum U test)

(ns not significant, DH double parental heredity, SH single parental heredity, MH maternal heredity, PH paternal heredity, TSG total study group)

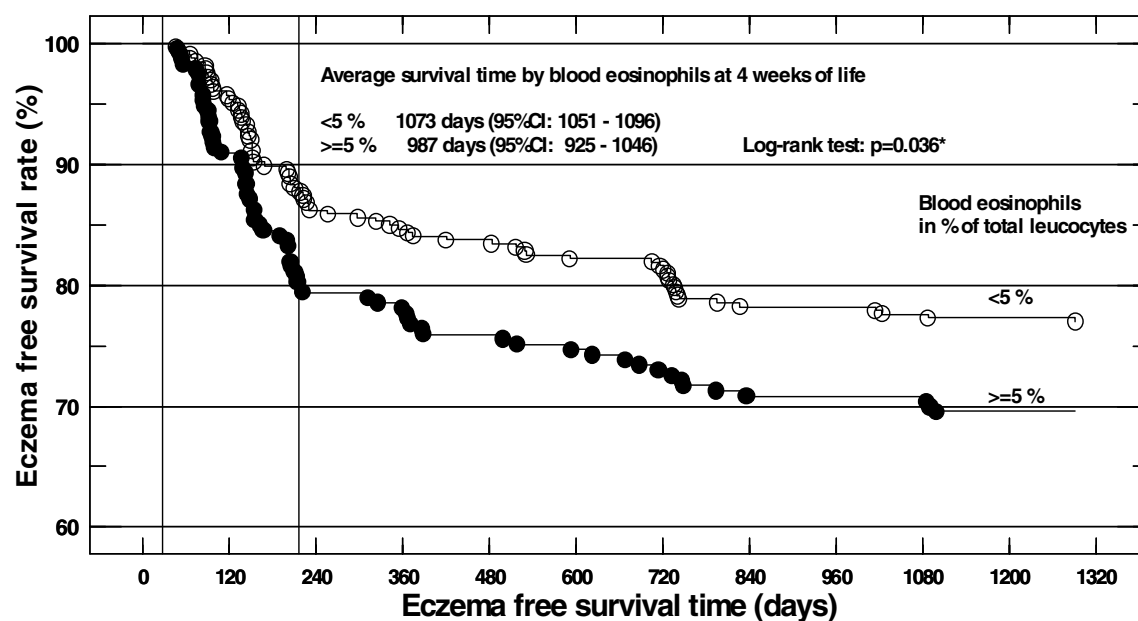
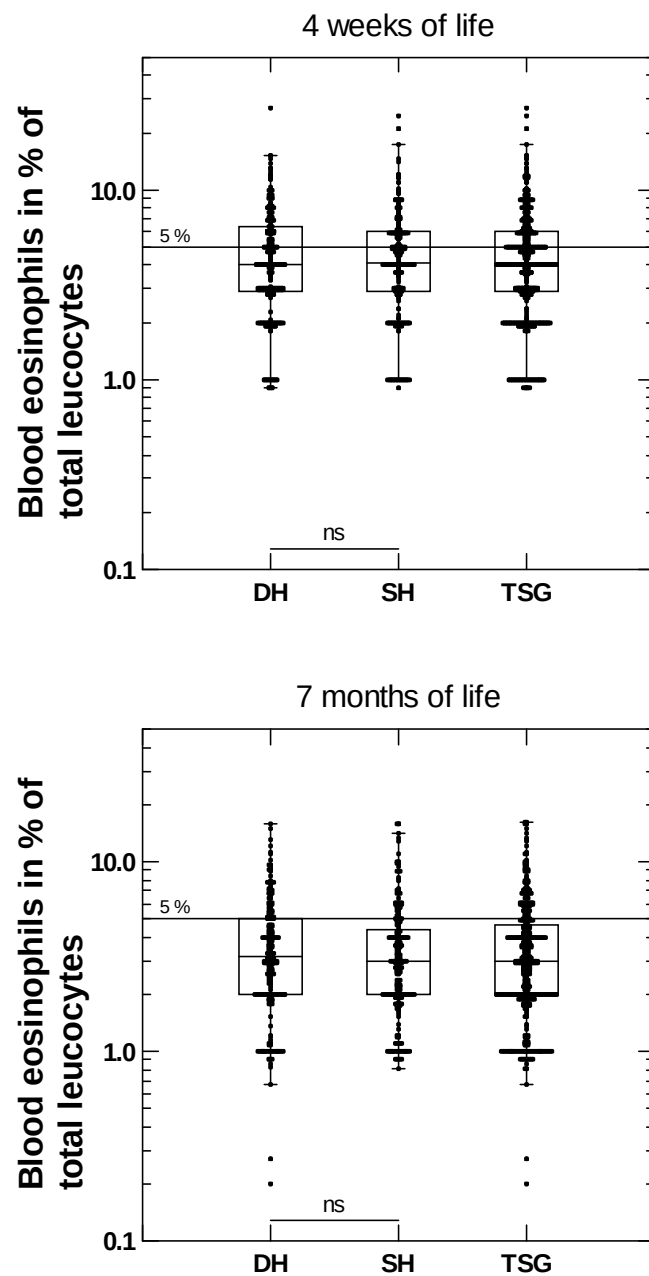


Fig 3 Eczema free survival rate (%) in infants for normal (thin) and elevated (thick black) blood eosinophils measured at age of 4 weeks until 1320 days of life. In infants with EE at 4 weeks of life, AD occurred approximately 100 days earlier (log-rank test: p=0.036)



Supplement Fig 1 Distribution of blood eosinophils in % for double heredity (DH), single heredity (SH) and in the total study group (TSG) (Mann-Whitney U-Tests between subgroups)