

Prevalence of type I sensitization to alpha-gal in forest service employees and hunters: Is the blood type an overlooked risk factor in epidemiological studies of the α -Gal syndrome?

To the Editor

We read with great interest the cross-sectional study presented by Fischer et al.¹ showing the higher risk to develop red meat allergy in individuals exposed to ticks and with a high prevalence of anti- α -Gal IgE response. The α -Gal syndrome is a tick-related allergy, affecting individuals mainly in Europe, the United States, and Australia. The sensitization mediated by the IgE response to tick-produced proteins with α -Gal modifications could result in anaphylactic reactions to tick bite and/or red meat consumption.^{2,3} Fischer and colleagues¹ characterized the risk factors associated with the α -Gal syndrome in forest service employees and hunters from Germany. They found that tick bites and high levels of anti- α -Gal IgE were important risk factors associated with the α -Gal syndrome in these individuals, a common finding in other similar studies.⁴ Other risk factors such as gender, pollen allergy, bronchial asthma, pet-keeping, age and lifestyle, among others, have also been considered in studies on the epidemiology of the α -Gal syndrome.^{3,4} However, factors

affecting directly the capacity of the immune system to produce anti- α -Gal antibodies are not usually included in these studies.

We propose that all factors increasing the capacity of the immune system to produce anti- α -Gal antibodies should be considered as a risk factor to develop the α -Gal syndrome. It was previously shown that individuals with blood type B produce less anti- α -Gal IgE antibodies, and red meat allergy after tick bites is strongly associated with individuals lacking the blood type B.³ In agreement with these findings, the frequency of blood group B in Europe, the United States, and Australia where the α -Gal syndrome is increasingly being reported is lower than in Africa and Asia where reports of this allergy are scarce (Figure 1). The presence of blood group B will reduce the capacity of the immune system to produce anti- α -Gal antibodies, presumably due to tolerance to α -Gal, which is very similar to blood group B antigen.^{2,5} These results support that the allergy to α -Gal should also be considered in the context of the susceptibility to infectious diseases caused by pathogens with α -Gal on their surface.⁵ High anti- α -Gal IgG and IgM antibody levels were associated with protection against malaria.⁶ Remarkably, the frequency of blood group B was positively correlated to the incidence of malaria and tuberculosis in endemic regions.⁵

Therefore, individuals with blood group B and reduced levels of anti- α -Gal antibodies should have lower risk to develop the α -Gal syndrome, but will have the trade-off of higher susceptibility to infectious diseases that are controlled by the immune system through anti- α -Gal immunity. We believe that this reasoning and evidence justify the inclusion of blood groups in the analysis of risk factors and susceptibility to the α -Gal syndrome.

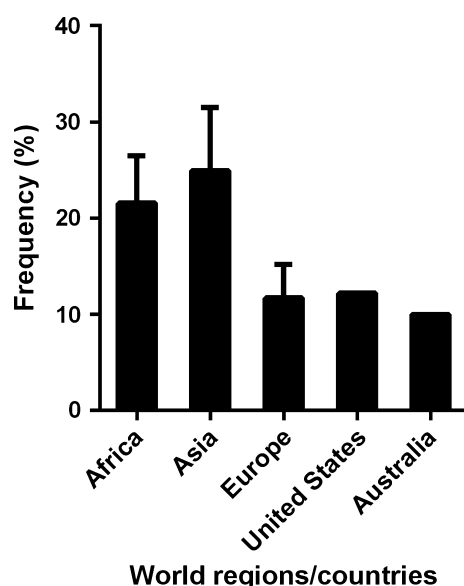


FIGURE 1 Worldwide frequency of blood group B. The mean of blood group B frequency in Africa, Asia, and Europe as well as the frequency of this blood group in the United States and Australia is shown. Individuals with blood type B have antibodies against antigen A, but not against self-antigen B, which is very similar to α -Gal. Data were collected from Cabezas-Cruz et al.⁵

CONFLICT OF INTEREST

All authors contributed equally to this correspondence and there is no conflict of interest.

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REFERENCES

1. Fischer J, Lupberger E, Hebsaker J, et al. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters. *Allergy*. 2017;72:1540-1547.
2. Mateos-Hernández L, Villar M, Moral A, et al. Tick-host conflict: immunoglobulin E antibodies to tick proteins in patients with anaphylaxis

to tick bite. *Oncotarget*. 2017;8:20630-20644. [http://www.impactjournals.com/oncotarget/index.php?journal=oncotarget&page=article&op=view&path\[\]=15243&path%5B%5D=48742](http://www.impactjournals.com/oncotarget/index.php?journal=oncotarget&page=article&op=view&path[]=15243&path%5B%5D=48742)

3. Hamsten C, Tran TA, Starkhammar M, et al. Red meat allergy in Sweden: association with tick sensitization and B-negative blood groups. *J Allergy Clin Immunol*. 2013;132:1431-1434.
4. Villalta D, Pantarotto L, Da Re M, et al. High prevalence of sIgE to Galactose- α -1,3-galactose in rural pre-Alps area: a cross-sectional study. *Clin Exp Allergy*. 2016;46:377-380.
5. Cabezas-Cruz A, Mateos-Hernández L, Alberdi P, et al. Effect of blood type on anti- α -Gal immunity and the incidence of infectious diseases. *Exp Mol Med*. 2017;49:e301.
6. Yilmaz B, Portugal S, Tran TM, et al. Gut microbiota elicits a protective immune response against malaria transmission. *Cell*. 2014;159:1277-1289.

Reply

To the Editor:

In our recent paper entitled "Prevalence of type I sensitization to alpha-gal in forest service employees and hunters," we described tick bites and elevated total IgE as major risk factors for type I sensitization to the carbohydrate galactose- α -1,3-galactose (alpha-gal).¹ Cabezas-Cruz and de la Fuente suggested in their letter to the editor the need to evaluate the blood type in epidemiological studies regarding alpha-gal.² Humans cannot synthesize alpha-gal based on a lack of the enzyme α -1,3-galactosyl-transferase.³ Therefore, alpha-gal is immunogenic and type I sensitization to alpha-gal is underlying the alpha-gal syndrome with immediate type and delayed immediate type reactions to mammalian innards and meat.⁴ The human blood group B antigen represents a fucosylated galactose- α -1,3-galactose structure, and based on similarities to alpha-gal, a susceptibility to immune tolerance to alpha-gal was hypothesized for individuals with blood groups B and AB (B blood types). Indeed, a recent study by Hamsten et al. suggests reduced type I sensitization to alpha-gal in individuals with B blood types.⁵ Therefore, we appreciate the correspondence of Cabezas-Cruz and de la Fuente and agree that investigating the human blood type may be important to assess potential risk factors also in the population investigated by us.

The analysis of blood type characteristics of serum using defined erythrocytes in a haemagglutination test was established and approved by the Ethics Commission of the Medical Faculty of the University of Tuebingen.^{1,4} Z-differences were used to compare the data with the blood type distribution of the German general population (link: www.blutspendedienst.com). Surplus serum of N=298/300 participants from our study among forest service employees and

hunters was examined for blood type characteristics in the serum. As indicated by the z-differences, blood type O was more frequent and blood types B and AB were less frequently detected in our study as expected to their distribution in the German general population (Table 1A). Subanalyses with comparison of blood type patterns of participants with and without type I sensitization to alpha-gal showed equal distribution (z-difference ≤ 1.5) of blood types between both subgroups, including blood group B (fisher exact test $P=0.52$) (Table 1B). In this epidemiological study, most of the participants with alpha-gal sIgE positivity were sensitized but had no type I allergy to mammalian meat. Remarkably, analyzing a case series of patients with alpha-gal syndrome (surplus serum of N=33/55 patients), we also found blood type B being similarly represented with 9.1% compared to 11% in the general population (Table 1C).⁴ Next, we analyzed alpha-gal sIgE and total IgE (tIgE) levels in alpha-gal sIgE-positive study participants only and compared the data from non-B blood types (n=96) data from participants with B blood types (n=9). Mean alpha-gal sIgE in participants with non-B blood types was 0.45 kU/L (quartiles 0.18 kU/L/2.05 kU/L), and mean tIgE was 103 kU/L (quartiles 52.7 kU/L/235.5 kU/L). Mean alpha-gal sIgE in participants with B blood types was 0.30 kU/L (quartiles 0.12 kU/L/1.08 kU/L), and mean tIgE was 48.4 kU/L (quartiles 29.1 kU/L/178.0 kU/L). Consequently, no significant differences for sIgE levels and tIgE levels were found between these subgroups (sIgE, Mann-Whitney $P=0.38$; tIgE, Mann-Whitney $P=0.20$). We actually recalculated the binary logistic regression of our study as suggested by Cabezas-Cruz and de la Fuente and included the variable "B blood types" as additional risk factor (Online repository S1).¹ The detection of the B blood type did not significantly decrease the risk of alpha-gal sIgE positivity (adjusted OR 0.792, CI 0.323-1.944; $P=0.61$), which challenges previous interpretations.

Limitations of our study are that blood type characteristics were obtained from serum by examining isoagglutinins, blood type-

Abbreviations: Alpha-gal, galactose- α -1,3-galactose; CI, confidence interval; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; OR, odds ratio; sIgE, specific IgE; tIgE, total IgE

This work was supported in part by a research grant awarded to JF from ThermoFisher Scientific, ImmunoDiagnostics (formerly Phadia) in Freiburg, Germany.

TABLE 1 (A) Comparison of the blood type distribution of participants of the epidemiological study by Fischer et al. (Ref. 1) with the of blood type distribution in the German general population (link: www.blutspendedienst.com). The distribution of blood groups of the German general population is derived from blood typing with erythrocytes, and the study-related data are based on the examination of blood type characteristics of serum using defined erythrocytes in a haemagglutination test. (B) Subanalyses with comparison of blood type pattern of participants of the study with and without type I sensitization to alpha-gal. (C) Blood type distribution in a case series of patients with alpha-gal syndrome (Fischer et al., Ref. 4).

Blood type	Cross-sectional study with forest employees and hunters (Ref. 1)						Patients with alpha-gal syndrome (Ref. 4)
	A			B			C
	Alpha-gal study (n=298)	German general population	z-difference	Alpha-gal-positive (n=105)	Alpha-gal-negative (n=193)	z-difference	Alpha-gal syndrome case series (n=33)
O	46.6%	41%	1.94	50.5%	44.6%	0.95	60.6%
A	43%	43%	0	41%	44%	-0.50	30.3%
B	8.4%	11%	-1.62	6.7%	9.3%	-0.81	9.1%
AB	2.0%	6%	-4.93	1.9%	2.1%	-0.11	0%

associated IgM and IgG antibodies, with defined erythrocytes in a haemagglutination test. Titers of isoagglutinins are independent from the genetically determined expression of blood type antigen and can be influenced by various individual and environmental factors.⁶ False-positive results can occur due to irregular antibodies in the serum or unspecific agglutination and false-negative results due to low isoagglutinins titers in the serum or degradation of blood type antigen on test erythrocytes due to cell aging.^{6,7} Therefore, this test does not carry out the same specificity and sensitivity as blood typing with erythrocytes. As a consequence, the presented data suggest rather underrepresentation of B blood types in comparison with German general population. The equal distribution of B blood types between study groups strongly argues that the blood group is not that relevant for susceptibility to develop type I sensitizations to alpha-gal as previously assumed. Already in the 1980s, Galili et al. studied the characteristics of mammalian alpha-gal epitopes and the closely related human blood group B antigen.⁸ Interestingly, the single branched fucose of the human blood group B antigen results in a significant structural modification of the alpha-gal epitope.⁸ Due to this modification, individuals with B blood types exhibit natural anti-alpha-gal IgG antibodies equivalent to non-B blood types, indicating a high degree of specificity of human anticarbohydrate antibodies.⁸ Our data indicate that comparable characteristics can be expected for alpha-gal sIgE, but further studies are warranted.

In conclusion, we could not observe any evidence in our studies that individuals with B blood types carry a reduced susceptibility to type I sensitization to alpha-gal, reduced alpha-gal sIgE levels, or reduced risk for the development of the alpha-gal syndrome.

AUTHOR CONTRIBUTION

J. Fischer wrote this reply in close collaboration with all the authors. The presented data are part of the doctoral theses of J. Hebsaker.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Fischer J, Lupberger E, Hebsaker J, et al. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters. *Allergy*. 2017;72:1540-1547.
2. Cabezas-Cruz A, de la Fuente J. Prevalence of type I sensitization to alpha-gal in forest service employees and hunters: is the blood type an overlooked risk factor in epidemiological studies of the alpha-gal syndrome. *Allergy*. 2017;72:2044-2045.

3. Galili U. Anti-Gal: an abundant human natural antibody of multiple pathogeneses and clinical benefits. *Immunology*. 2013;140:1-11.
4. Fischer J, Yazdi AS, Biedermann T. Clinical spectrum of alpha-gal syndrome: from immediate type to delayed immediate type reactions to mammalian innards and meat. *Allergo J Int*. 2016;25:55-62.
5. Hamsten C, Tran TAT, Starkhammar M, et al. Red meat allergy in Sweden: association with tick sensitization and B-negative blood groups. *J Allergy Clin Immunol*. 2013;132:1431-1434.
6. Lehner B, Eichelberger B, Jungbauer C, Panzer S. The blood group a genotype determines the level of expression of the blood group a on platelets but not the anti-B isotiter. *Transfus Med Hemother*. 2015;42:366-371.
7. Baehr EA, Kretschmer V. Zuverlässigkeit der Blutgruppenbestimmung bei Neugeborenen. *Lab. Med*. 1989;10:134-138.
8. Galili U, Buehler J, Shohet SB, Macher BA. The human natural anti-Gal IgG. III. The subtlety of immune tolerance in man as demonstrated by crossreactivity between natural anti-Gal and anti-B antibodies. *J Exp Med*. 1987;165:693-704.

SUPPORTING INFORMATION

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