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Skin testing improves predictive value of mid-range peanut specific IgE and Ara h 2 levels in the LEAP study

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Clinical Implications:

There is a need to easily predict clinically important peanut allergy without OFC. The presence of both a positive SPT (3mm) and Ara h 2-sIgE ≥0.29 kU/L at 60 months may preclude the need for OFC in children meeting LEAP study inclusion criteria.

Peanut allergy has a prevalence of ~2% and is the leading cause of food-induced fatal and near-fatal anaphylaxis. [1] Distinguishing between sensitization and clinically relevant peanut allergy remains challenging, especially for patients whose peanut-specific immunoglobulin E (PN-sIgE) levels are neither very low nor very high.

PN-sIgE is the most sensitive test for prediction of clinically important peanut allergy (sensitivity (SN)=0.93), whereas Ara h 2-sIgE is the most specific (specificity (SP)=0.92). [2] Ara h 2 is the best overall individual diagnostic test by receiver operative characteristic (ROC) analysis, with an area-under-the-curve (AUC) of 0.84, compared to peanut-sIgE, which has an AUC of 0.69. [2]

Historically, PN-sIgE ≥0.35 kU/L was reported as a positive result, with low specificity (23-58%) and positive predictive value (PPV) (44-71%).[3] Some laboratories now report PN-sIgE of ≥0.10 kU/L as a positive test but the value of this cut-off has not been well studied.

Accurately predicting peanut allergy improves with higher peanut and Ara h 2-sIgE values. Previous data suggest a PN-sIgE of 6 kU/L has a 90% predicted probability of clinical reactivity and at 13 kU/L this probability increases to 95% with a sensitivity of 60%, specificity of 96%, PPV of 99% and negative predictive value (NPV) of 35%. [4] A peanut skin prick wheal size of ≥8 mm and PN-sIgE ≥15 kU/L are reported to predict peanut allergy with 95% and 92% certainty for a positive challenge, respectively. [5] For Ara h 2-sIgE, a cutoff of ≥1.75 kU/L has a 100% positive predictive value, and ~30% of patients could be diagnosed with 100% accuracy.[6] The addition of other measurements such as skin prick test (SPT) wheal size or ex vivo basophil reactivity further increases the accuracy of these tests.[7, 8]

The Learning about Early Peanut Allergy (LEAP) trial was pivotal in demonstrating the benefit of early introduction of peanuts in high-risk children to decrease the frequency of peanut allergy.[9] Santos et al. used this data set to identify severity and threshold of reactions during oral peanut challenges.[7] Optimal cutoffs conferring high risk for developing severe allergic reactions included BAT 48%, Ara h 2-sIgE of 1.4 kU/L, PN-sIgE >5 kU/L and a peanut SPT of 8mm. Multivariate models were superior to individual biomarkers and were used to calculate the probability of serious adverse events during oral food challenge (OFC). [7]

The LEAP study team developed a model using SPT, PN-sIgE, Ara h 1, Ara h 2, and Ara h 3-sIgE values to predict peanut allergy in the absence of an OFC. [8] This prediction model, applied to 617 LEAP participants with a determinate OFC, had an AUC of 0.99, with an overall error rate of 2.6%. However, mismatches between observed and predicted OFC results occurred when PN-sIgE ranged from 0.20-2.17 kU/L. [8]

There remains a need to develop simple approaches using easily obtainable biomarkers to predict the presence of clinically important allergies to peanuts without performing OFC in subjects with PN-sIgE values in the non-extreme range of ≥0.1 and <15 kU/L.

To address this need, we focused on the peanut avoidance group (n=321) in the LEAP study data set because this group is a cohort of subjects at highest risk of having demonstratable peanut allergy at 5 years (60 months) of age when the graded OFC was performed.

All LEAP study participants randomized to the peanut avoidance group with very low PN-sIgE<0.1 or very high PN-sIgE >9 kU/L at 60 months (n= 186) had predictable outcomes of either negative or positive OFC respectively (Figure E1). Further biomarkers are not needed as the PN-sIgE data gave an AUC 1.0 with 100% sensitivity, specificity and accuracy. Changing the upper limit to >13kU/L or >15 kU/L based on the published literature did not affect this finding.

For subjects in the peanut avoidance group with PN-sIgE in a non-extreme range ≥0.1 to ≤9 kU/L at 60 months, a positive SPT at 60 months, defined as ≥3mm, was superior (orange line, AUC 0.8619, $P<10^{-11}$, n=116) to PN-sIgE (yellow line, AUC= 0.67, P= 0.002, n= 116) or Ara h 2-sIgE alone at 60 months (green line, AUC= 0.86, $p<10^{-8}$, n=112) (Figure 1). These subjects have "difficult to diagnose" peanut allergy, and additional biomarkers are needed to make more accurate predictions. Sensitivity, specificity, accuracy, PPV, and NPV are shown for optimal cutoffs of PN-sIgE =6 kU/L (selected for PPV ≥90%) and for Ara h 2-sIgE =0.56 kU/L (selected for PPV=100%).

The presence of a positive SPT ≥3mm at 60 months markedly increases diagnostic accuracy of PN-sIgE (AUC 0.8653, $p=10^{-9}$, n=116) but, at the chosen threshold of 6 kU/L, did not change the performance metrics (blue line, Figure 2). However, for Ara h 2-sIgE, the presence of a positive SPT not only increased diagnostic accuracy (purple line, AUC 0.943, $p <10^{-12}$, n = 112), but also allowed a lower threshold of Ara h 2-sIgE (≥0.29 kU/L) to reach 100% SP and PPV (no false positives) (Figure 1). A flowsheet of this approach is shown (Figure E2). Importantly, as the SPT wheal size increases, the cut off for Ara h 2-sIgE to achieve 100% SP and 100% PPV decreases dramatically (Figure 2).

In conclusion, the presence of a positive SPT and an Ara h 2-sIgE of ≥0.29 may preclude the need for an OFC in subjects who meet the LEAP study inclusion criteria [9], with the understanding that there is a significant false negative rate of 52%. Compared with Sever et al (8) where analysis of multiple parameters in all subjects regardless of PN-sIgE values led to an error rate of 2.8%, our overall error rate using only Ara h 2-sIgE and the presence of a positive SPT was 12.5%.

Strengths of this report include the focus on subjects with difficult to diagnose peanut allergies and the use of easy to obtain clinical data. Limitations include the makeup of the LEAP cohort, the retrospective analysis, the relatively small number of subjects in the difficult to diagnose range of PN-sIgE with positive SPT, and the need to repeat this study both prospectively and with a different database.

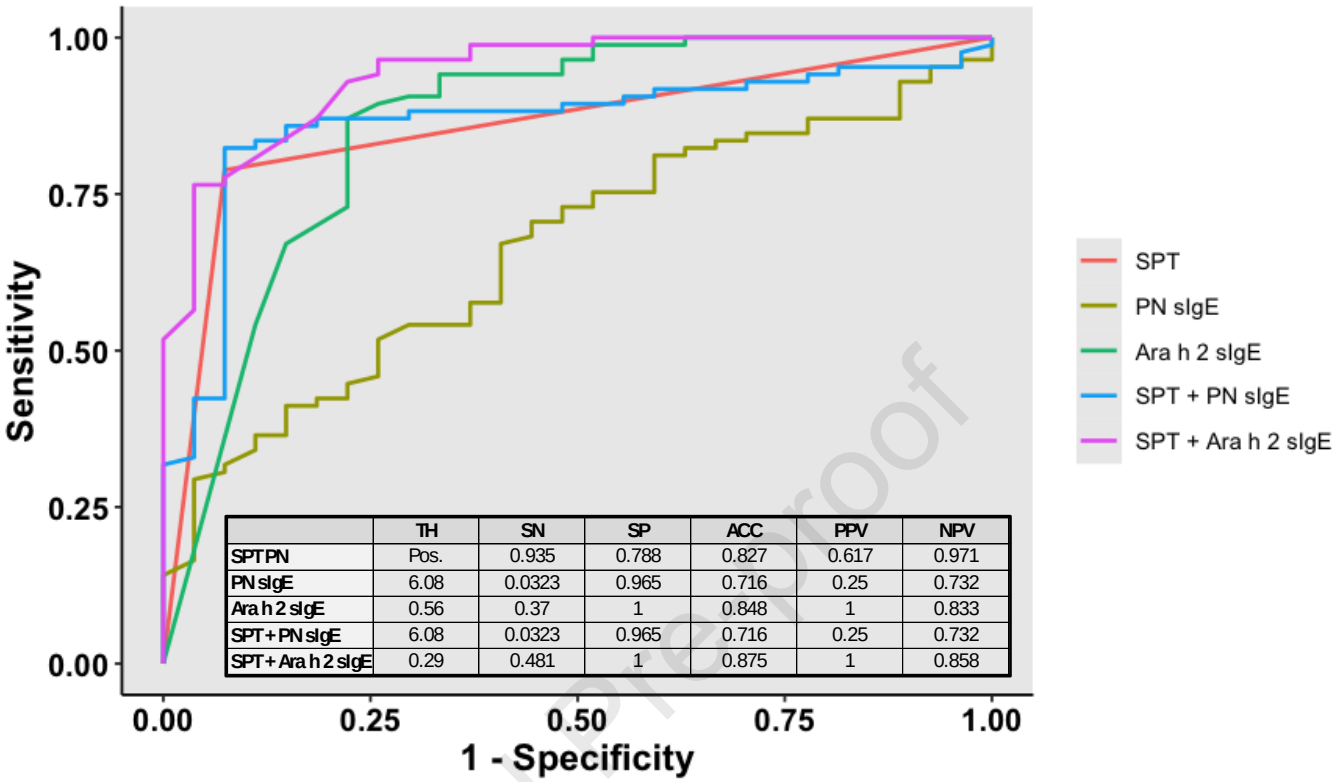
References:

1. Warren C, Lei D, Sicherer S, Schleimer R, Gupta R. Prevalence and characteristics of peanut allergy in US adults. J Allergy Clin Immunol. 2021 Jun;147(6):2263-2270.e5
2. Lieberman JA, Glaumann S, Batelson S, Borres MP, Sampson HA, Nilsson C. The utility of peanut components in the diagnosis of IgE-mediated peanut allergy among distinct populations. J Allergy Clin Immunol Pract. 2013 Jan;1(1):75-82.
3. Stiefel G, Anagnostou K, Boyle RJ, Brathwaite N, Ewan P, Fox AT, et al. BSACI guideline for the diagnosis and management of peanut and tree nut allergy. Clin Exp Allergy. 2017 Jun;47(6):719-39.
4. Maloney JM, Rudengren M, Ahlstedt S, Bock SA, Sampson HA. The use of serum-specific IgE measurements for the diagnosis of peanut, tree nut, and seed allergy. J Allergy Clin Immunol. 2008 Jul;122(1):145-51
5. Roberts G, Lack G. Diagnosing peanut allergy with skin prick and specific IgE testing. J Allergy Clin Immunol. 2005 Jun;115(6):1291-6.
6. Kansen HM, van Erp FC, Knulst AC, Ehlers AM, Lyons SA, Knol EF, et al. Accurate Prediction of Peanut Allergy in One-Third of Adults Using a Validated Ara h 2 Cutoff. J Allergy Clin Immunol Pract. 2021 Apr;9(4):1667-1674.e3.
7. Santos AF, Du Toit G, O'Rourke C, Becares N, Couto-Francisco N, Radulovic S, et al. Biomarkers of severity and threshold of allergic reactions during oral peanut challenges. J Allergy Clin Immunol. 2020 Aug;146(2):344-55.
8. Sever ML, Calatroni A, Roberts G, du Toit G, Bahnson HT, Radulovic S, et al. Developing a Prediction Model for Determination of Peanut Allergy Status in the Learning Early About Peanut Allergy (LEAP) Studies. J Allergy Clin Immunol Pract. 2023 Jul;11(7):2217-2227.e9.
9. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med. 2015 Feb 26;372(9):803-13.

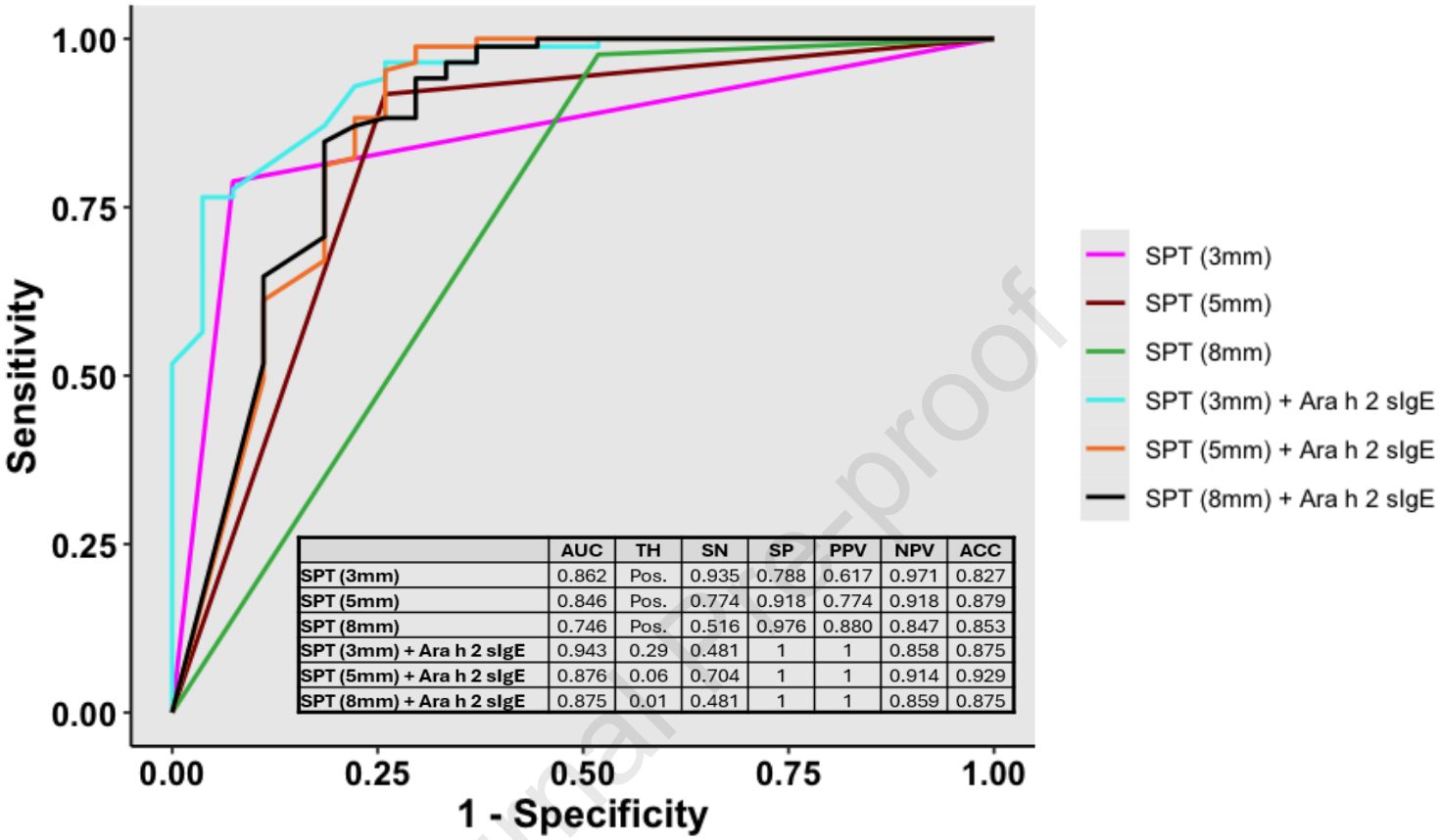
Figure legends

Figure 1. A positive SPT (3mm) augments prediction of peanut allergy when PN-sIgE ≥0.1 and ≤9 kU/L at 60 months. TH=threshold, SN=sensitivity, SP=specificity, ACC=accuracy, PPV=positive predictive value, NPV=negative predictive value.

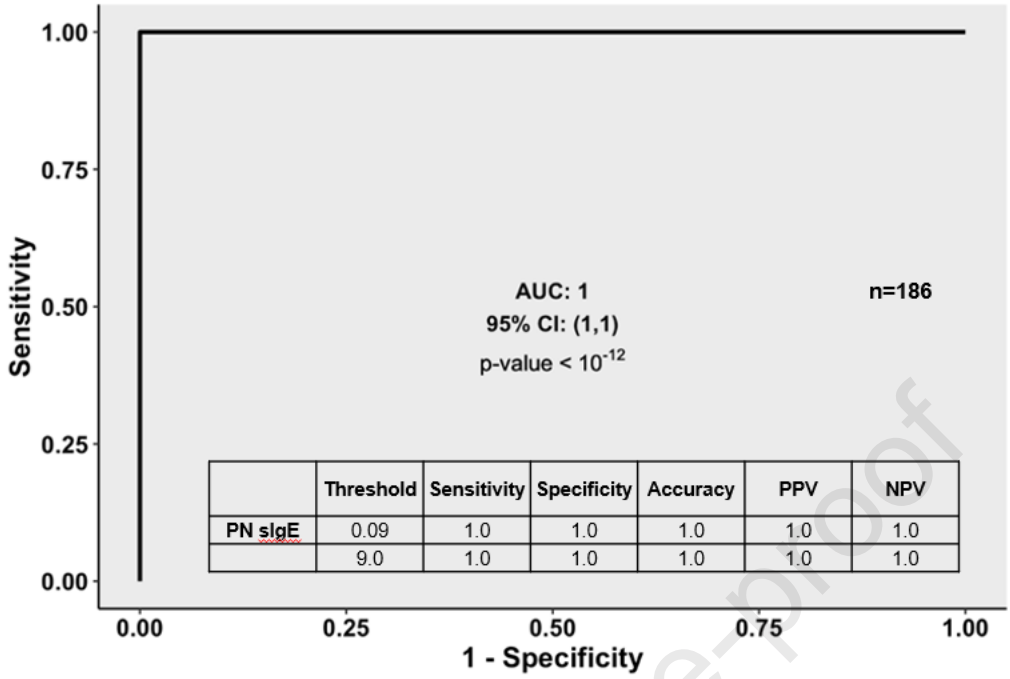
Figure 2. Increasing SPT size from 3mm (n=43) to 5mm (n=27) to 8mm (n=15) decreases the threshold of Ara h 2-sIgE needed to achieve 100% SP and PPV.



	TH	SN	SP	ACC	PPV	NPV
SPT PN	Pos.	0.935	0.788	0.827	0.617	0.971
PN sIgE	6.08	0.0323	0.965	0.716	0.25	0.732
Ara h 2 sIgE	0.56	0.37	1	0.848	1	0.833
SPT + PN sIgE	6.08	0.0323	0.965	0.716	0.25	0.732
SPT + Ara h 2 sIgE	0.29	0.481	1	0.875	1	0.858



	AUC	TH	SN	SP	PPV	NPV	ACC
SPT (3mm)	0.862	Pos.	0.935	0.788	0.617	0.971	0.827
SPT (5mm)	0.846	Pos.	0.774	0.918	0.774	0.918	0.879
SPT (8mm)	0.746	Pos.	0.516	0.976	0.880	0.847	0.853
SPT (3mm) + Ara h 2 sIgE	0.943	0.29	0.481	1	1	0.858	0.875
SPT (5mm) + Ara h 2 sIgE	0.876	0.06	0.704	1	1	0.914	0.929
SPT (8mm) + Ara h 2 sIgE	0.875	0.01	0.481	1	1	0.859	0.875



	Threshold	Sensitivity	Specificity	Accuracy	PPV	NPV
PN sIgE	0.09	1.0	1.0	1.0	1.0	1.0
	9.0	1.0	1.0	1.0	1.0	1.0

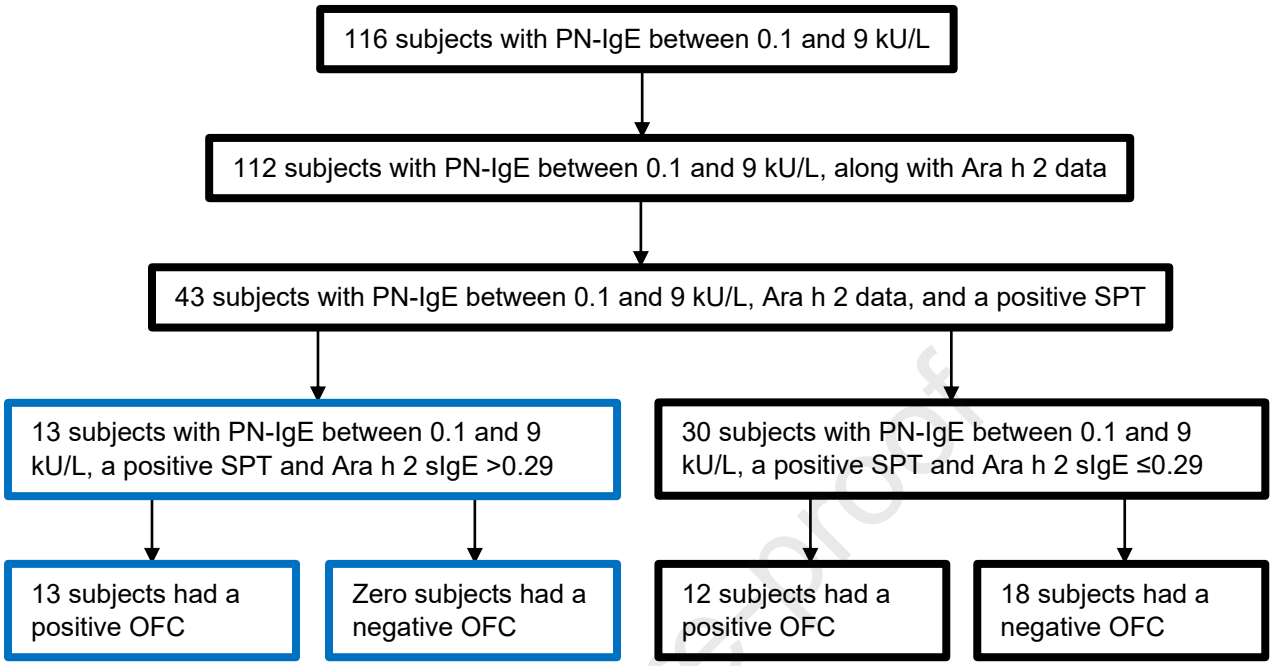


Figure E1. Peanut allergy is easy to predict in peanut avoidance group with PN-sIgE <0.1 or >9 kU/L at 60 months.

Figure E2. Flowchart. Key findings are highlighted in blue.