Allergy

WILEY

Allergy EUROPEAN JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY EAACI

LETTER OPEN ACCESS

IgG Removal Enhances the Sensitivity of Amoxicillin-Specific IgE Detection in ImmunoCAP and Radioallergosorbent Test

Jose A. Céspedes[1,2] | Ana M. Pérez-Moreno[1,2] | Raquel Jurado-Escobar[1] | Gador Bogas[1,3,4] | María Salas[1,3,4] | Adriana Ariza[1,3,4] | Rosa Muñoz-Cano[4,5,6] | Cristobalina Mayorga[1,3,4] | María Jose Torres[1,2,3,4] | Juan L. Paris[1,4]

[1]IBIMA-Plataforma BIONAND, Malaga, Spain | [2]Universidad de Málaga, Malaga, Spain | [3]Hospital Regional Universitario de Málaga, Malaga, Spain | [4]RICORS "Enfermedades Inflamatorias", Spain | [5]Hospital Clínic, Universitat de Barcelona, Barcelona, Spain | [6]Clinical and Experimental Respiratory Immunoallergy, Institut Investigacions Biomediques August Pi I Sunyer (IDIBAPS), Barcelona, Spain

Correspondence: Juan L. Paris (juan.paris@ibima.eu)

Received: 19 February 2025 | **Revised:** 24 March 2025 | **Accepted:** 7 May 2025

Funding: This study was supported by an Innovation Grant from the Andalusian Regional Ministry of Health (PIN-0113-2020) and by Institute of Health "Carlos III" (ISCIII) of the Ministry of Economy and Competitiveness (MINECO) (grant co-funded by European Regional Development Fund, RICORS REI RD21/0002/0008, RD21/0002/0058, PI18/00095, PI21/00329). C.M. holds "Nicolas Monardes" research contract by Andalusian Regional Ministry Health (grant no. RC-0004-2021). J.L.P. acknowledges grant RYC2021-034536-I funded by MCIU/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR. A.P.M. acknowledges PhD fellowship PREP2022-000567 funded by CIN/AEI/10.13039/501100011033 and by ESF+. J.A.C.L. holds a P-FIS contract (FI22/00199) by ISCIII. TEM experiments were performed in the ICTS "NANBIOSIS", more specifically in the U28 Unit at IBIMA Plataforma BIONAND. Funding for open access charge: Universidad de Málaga/CBUA.

To the Editor,

β-Lactam (BL) antibiotics are widely prescribed but frequently cause hypersensitivity reactions (DHRs) [1], particularly IgE-mediated allergies, ranging from mild skin reactions to life-threatening anaphylaxis. Traditional diagnostic methods, such as skin and drug provocation tests, are invasive and risky. In vitro tests like immunoassays for detecting serum-specific IgE (sIgE) offer a safer alternative but show low sensitivity and moderate specificity [2, 3], often leading to false diagnoses [4, 5].

The limited sensitivity of these tests may be due to: (i) the small size of drug molecules, which require covalent binding to a carrier protein to elicit an immune response [4], (ii) incomplete interaction with the immunological system inducing low sIgE levels, and (iii) high IgG concentrations that block IgE binding to the solid phase [6] even though some studies have reported that they do not affect certain techniques, such as ImmunoCAP [7]. Therefore, we hypothesized that IgG removal from serum could enhance sIgE detection by increasing its binding capacity to hapten-carrier conjugates in immunoassays.

Mesoporous silica nanoparticles (MSNs) are a promising platform for biomolecule capture due to their large surface area, tunable pore sizes, and ease of chemical modification. Previously, we demonstrated that IgG removal by using protein G′-grafted MSNs (MSN-pG′) improved sIgE detection for various allergens/haptens [8]. In patients with confirmed amoxicillin (AX) allergies, ImmunoCAP sensitivity increased from 12.5% to 37.5%.

This study evaluated MSN-pG′ pre-treatment of sera in a larger cohort of BL-allergic patients and controls using ImmunoCAP and radioallergosorbent test (RAST). MSN synthesis and characterization confirmed successful protein G′ grafting, with optimal 12 nm pore size, particle diameter of 149.3 ± 48.4 nm, Zeta potential of −26.3 ± 8.7 mV, and specific infrared bands confirming protein presence (Figure S1). Serum samples (300 μL) were treated with 6 mg of MSN-pG′ for 1 h at room temperature, followed by centrifugation for nanoparticle removal.

We analyzed sera from 24 BL-allergic patients (following EAACI guidelines [4]) and 11 tolerant controls before and after

Jose A. Céspedes and Ana M. Pérez-Moreno contributed equally to this work as co-first authors.

María Jose Torres and Juan L. Paris contributed equally to this work as co-last authors.

TABLE 1 | Allergological workups, in vivo and in vitro results of AX-allergic patients and tolerant controls.

						Skin test					DPT	Immunoassays			
ID	Comorbidities	Suspected drug	Clinical symptoms	Time interval between reaction and study (days)	Time interval between drug intake and reaction (min)	AX	BP-OL	MDM	AX-CLV	CLV	AX	AX-sIgE CAP pre-MSN (kUA/L)	AX-sIgE CAP post-MSN (kUA/L)	%RAST AX pre-MSN	%RAST AX post-MSN
Pt.1	No	AX	Urt/AE – I	180	15	Positive ID	Negative	Negative	ND	ND	ND	**0.74**	**2.23**	**45.94**	**46.60**.
Pt. 2	Yes	Piper-Tazo	Urt/AE – I	30	5	Negative	Positive prick	Negative	ND	ND	ND	**2.64**	**3.93**	**25.25**	**23.16**
Pt.3	No	AX-CLV	Urt/AE – I	28	2	Positive prick	Negative	Negative	Negative	ND	ND	**0.95**	**4.13**	**24.58**	**20.53**
Pt.4	No	AX-CLV	II	150	60	Positive ID	Negative	Negative	ND	ND	ND	**1.55**	**1.73**	**16.02**	**17.58**
Pt.5	Yes	AX-CLV	Urt/AE – I	60	5	Positive ID	Negative	Negative	ND	Negative	ND	0.05	0.07	**4.96**	**5.82**
Pt.6	No	AX	III	210	30	Positive ID	Negative	Negative	ND	ND	ND	0.24	**0.36**[a]	**4.65**	**7.57**
Pt.7	Yes	AX-CLV	III	150	90	Positive ID	Positive ID	Positive ID	ND	Negative	ND	0.003	0.02	**4.62**	**5.75**
Pt.8	Yes	AX-CLV	III	150	20	Positive ID	Negative	Negative	Negative	ND	ND	0.27	**0.35**[a]	**4.18**	**5.71**
Pt.9	No	AX-CLV	II	180	60	Positive ID	Negative	Negative	ND	Negative	ND	0.17	0.03	**4.15**	**4.07**
Pt.10	Yes	AX	Urt/AE – I	84	30	Negative	Positive ID	Positive ID	ND	ND	ND	0.29	**0.37**[a]	2.69	**4.53**[a]
Pt.11	No	AX-CLV	II	660	UNK	Positive prick	Negative	Negative	ND	Negative	ND	0.06	0.1	2.53	3.04
Pt.12	No	AX-CLV	III	300	15	Positive ID	Negative	Positive ID	ND	Negative	ND	0.01	0.02	2.47	2.35
Pt.13	Yes	AX	Urt/AE – I	9125	5	Positive ID	Negative	Negative	ND	Negative	ND	0.1	0.03	2.06	**3.36**[a]
Pt.14	No	AX	II	8395	15	Negative	Negative	Negative	ND	ND	Positive	0.07	0.01	1.68	2.41
Pt.15	Yes	AX	Urt/AE – I	7300	60	Positive ID	Negative	Negative	ND	Positive ID	ND	0.2	0.07	1.31	2.66
Pt.16	Yes	AX-CLV	Urt/AE – I	450	60	Positive ID	Negative	Negative	ND	Negative	ND	0.2	**0.41**[a]	0.10	1.31
Pt.17	No	AX-CLV	Urt/AE – I	270	10	Positive ID	Negative	Negative	ND	Negative	ND	0.04	0.04	0.48	0.82
Pt.18	No	AX-CLV	II	90	30	Positive ID	Negative	Negative	ND	Negative	ND	0.03	0.06	2.17	**3.87**[a]

(Continues)

TABLE 1 | (Continued)

ID	Comorbidities	Suspected drug	Clinical symptoms	Time interval between reaction and study (days)	Time interval between drug intake and reaction (min)	Skin test					DPT	Immunoassays			
						AX	BP-OL	MDM	AX-CLV	CLV	AX	AX-sIgE CAP pre-MSN (kUA/L)	AX-sIgE CAP post-MSN (kUA/L)	%RAST AX pre-MSN	%RAST AX post-MSN
Pt.19	No	AX	III	330	30	Positive prick	Negative	Negative	Negative	ND	ND	0.01	0.03	0.12	0.46
Pt.20	No	AX-CLV	III	60	10	Positive prick	Negative	Negative	ND	Positive ID	ND	0.06	0.15	2.23	2.03
Pt.21	No	AX	Urt/AE – I	4015	20	Positive ID	Negative	Negative	ND	ND	ND	0.02	0.06	1.24	2.12
Pt.22	No	AX-CLV	III	30	2	Positive prick	Negative	Negative	ND	Negative	ND	0.06	0.21	0.29	**19.35**[a]
Pt.23	Yes	AX	III	40	2	Positive prick	Negative	Negative	ND	ND	ND	0	0.03	2.02	1.01
Pt.24	No	AX-CLV	Urt/AE – I	720	15	Positive ID	Negative	Negative	ND	Negative	ND	0.07	0.13	0.52	1.27
Ctrl. 1	No	AX	II	150	20	Negative	Negative	Negative	ND	ND	Negative	0.01	0.03	1.33	2.65
Ctrl. 2	No	PV	II	22,995	UNK	Negative	Negative	Negative	ND	ND	Negative	0.08	0.1	1.29	2.40
Ctrl. 3	No	PV	Urt/AE – I	13,505	5	Negative	Negative	Negative	ND	ND	Negative	0.01	0.03	1.08	1.62
Ctrl. 4	No	CEF	Urt/AE – I	1460	30	Negative	Negative	Negative	ND	ND	Negative	0.03	0.03	2.04	1.68
Ctrl. 5	No	AX-CLV	Urt/AE – I	2920	UNK	Negative	Negative	Negative	ND	Negative	Negative	0.02	0.03	2.08	1.08
Ctrl. 6	No	AX	Urt/AE – I	240	180	Negative	Negative	Negative	ND	Negative	Negative	0.02	0.04	1.91	1.89
Ctrl. 7	No	PV	Urt/AE – I	13,870	5	Negative	Negative	Negative	ND	Negative	Negative	0.03	0.06	1.73	2.14
Ctrl. 8	No	PV	Urt/AE – I	4380	20	Negative	Negative	Negative	ND	ND	Negative	0.06	0.09	2.02	1.94
Ctrl. 9	No	AX	II	90	20	ND	Negative	Negative	ND	ND	Negative	0	0.04	1.18	1.42
Ctrl. 10	No	AX	II	150	60	Negative	Negative	Negative	ND	ND	Negative	0.07	0.11	1.65	1.72
Ctrl. 11	No	AX	II	164	30	ND	Negative	Negative	ND	ND	Negative	0.01	0.03	1.87	1.54

Abbreviations: AX, amoxicillin; BPO, benzylpenicilloyl; BP-OL, benzylpenicillin octa-L-lysine; CLV, clavulanic acid; ctrl, control; DPT, drug provocation test; grade II, moderate or anaphylaxis; grade III, severe or anaphylactic shock; ID, intradermal; MDM, minor determinant mixture; MSN, mesoporous nanoparticle; ND, not done; Pt, patient; sIgE, specific IgE; UNK, unknown.Urt/AE, urticaria-angioedema. Results in bold indicate value above positivity threshold.

[a]Indicates cases that become positive after treatment with MSN-pG′.

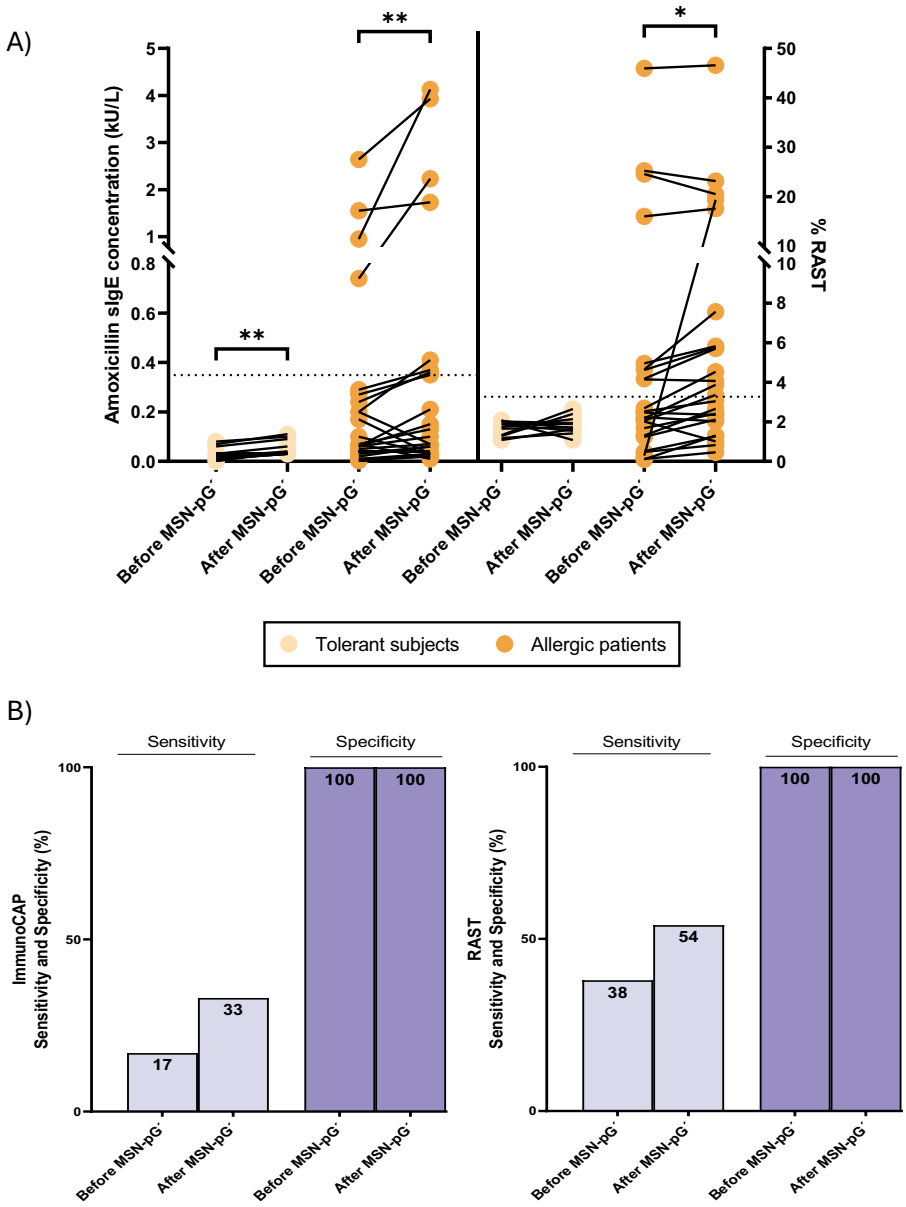

FIGURE 1 | (A) Effect of L-MSN-pG′ treatment before and after 1 h incubation in sera from confirmed amoxicillin-allergic patients ($n = 24$) in amoxicillin-specific IgE concentration and percentage of RAST. (B) Representation of MSN-pG′ treatment effect in the percentage of sensitivity and specificity of ImmunoCAP and RAST.

MSN-pG′ treatment using ImmunoCAP and RAST (Table 1, Figure 1A). AX-sIgE detection in ImmunoCAP followed manufacturer protocols, with positive results defined as ≥0.35 kU/L [9]. In RAST, AX was conjugated to poly-L-lysine and applied to a cellulose disc (further details can be found in Supporting Information), with positivity cutoffs determined by ROC curve analysis (Figure S2).

ImmunoCAP results showed a significant increase in AX-sIgE levels post-MSN-pG′ treatment in allergic patients ($p = 0.0085$) and controls ($p = 0.002$) (Figure 1A). However, only allergic patients had increased positive test rates, with sensitivity improving from 16.67% to 33.34%, while controls remained negative (100% specificity for both groups, Figure 1B). For RAST, MSN-pG′ significantly enhanced AX-sIgE detection only in allergic patients ($p = 0.01$) (Figure 1A), raising sensitivity from 37.5% to 54.16%, again with 100% specificity (Figure 1B).

Additionally, we measured total IgG and AX-sIgG captured by MSN-pG′ from sera. While captured total IgG levels showed no differences between groups, MSN-pG′ captured significantly higher AX-sIgG levels from allergic patients than tolerant controls ($p = 0.014$) (Figure S3). However, no correlation was found between captured IgG levels and serum sIgE detection improvement, showing that while the mechanism of improving drug-specific IgE detection does rely on IgG removal from the patient serum, the amount of IgG removed might not directly correlate with the enhancement of sIgE detection sensitivity. The time interval between reaction and study showed no significant influence on serum sIgE detection improvement either.

Overall, MSN-pG′ treatment significantly improved detection of sIgE to AX, converting 16% of previously negative cases to positive in both ImmunoCAP and RAST, and 30% when combining both tests. Notably, 57% of these newly positive cases had moderate or severe anaphylaxis reported in clinical history, underscoring the clinical relevance of this method. The increased sensitivity likely results from efficient IgG removal, which reduces competition for IgE binding sites, overcoming a key limitation of traditional immunoassays. This competition is especially relevant when IgE levels are low, such as in beta-lactam allergies, as previously demonstrated [8].

This novel approach enhances diagnostic accuracy while maintaining specificity, reducing the need for invasive in vivo tests. Implementing MSN-pG′ treatment prior to in vitro immunoassays (such as ImmunoCAP and RAST) could revolutionize allergy testing, confirming previous observations [8] but in a bigger cohort and not just in ImmunoCAP, providing safer and more reliable alternatives for patients with BL hypersensitivity. Further research should explore its application in other drug allergies and its integration into clinical practice.

Author Contributions

Conception and design J.L.P., C.M., and M.J.T.; data acquisition J.A.C., A.M.P.-M., R.J.-E., G.B., M.S., A.A., R.M., and J.L.P.; analysis of data J.A.C., A.M.P.-M., and J.L.P.; interpretation of data J.A.C., A.M.P.-M., J.L.P., and C.M.; writing original draft preparation J.A.C., A.M.P.-M., J.L.P., and C.M.; supervision J.L.P. and C.M. All authors have revised the manuscript and approved the version to be published.

Acknowledgments

This work was supported by an Innovation Grant from the Andalusian Regional Ministry of Health (PIN-0113-2020) and by Institute of Health "Carlos III" (ISCIII) of the Ministry of Economy and Competitiveness (MINECO) (grant co-funded by European Regional Development Fund, RICORS REI RD21/0002/0008, RD21/0002/0058, PI18/00095, PI21/00329). C.M. holds "Nicolas Monardes" research contract by Andalusian Regional Ministry Health (grant no. RC-0004-2021). J.L.P. acknowledges grant RYC2021-034536-I funded by MCIN/AEI/10.13039/501100011033 and by European Union NextGenerationEU/PRTR. A.M.P.-M acknowledges PhD fellowship PREP2022-000567 funded by CIN/AEI/10.13039/501100011033 and by ESF+. J.A.C. holds a P-FIS contract (FI22/00199) by ISCIII. TEM experiments were performed in the ICTS "NANBIOSIS", more specifically in the U28 Unit at IBIMA Plataforma BIONAND. Funding for open access charge: Universidad de Málaga/CBUA.

Conflicts of Interest

Cristobalina Mayorga, Maria Jose Torres, and Juan L. Paris are inventors on a patent application (patent application number: [WO2024023382A1]) related to the methods described in this manuscript. The rest of the authors declare that they have no conflicts of interest related to the content of this manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Jose A. Céspedes
Ana M. Pérez-Moreno
Raquel Jurado-Escobar
Gador Bogas
María Salas
Adriana Ariza
Rosa Muñoz-Cano
Cristobalina Mayorga
María Jose Torres
Juan L. Paris

References

1. A. Romano, M. Atanaskovic-Markovic, A. Barbaud, et al., "Towards a More Precise Diagnosis of Hypersensitivity to Beta-Lactams – An EAACI Position Paper," *Allergy* 75, no. 6 (2020): 1300–1315.

2. D. G. Ebo, J. Leysen, C. Mayorga, A. Rozieres, E. F. Knol, and I. Terreehorst, "The In Vitro Diagnosis of Drug Allergy: Status and Perspectives," *Allergy* 66, no. 10 (2011): 1275–1286.

3. C. Mayorga, G. E. Çelik, M. Pascal, et al., "Flow-Based Basophil Activation Test in Immediate Drug Hypersensitivity. An EAACI Task Force Position Paper," *Allergy* 79, no. 3 (2024): 580–600.

4. C. Mayorga, G. Celik, P. Rouzaire, et al., "In Vitro Tests for Drug Hypersensitivity Reactions: An ENDA/EAACI Drug Allergy Interest Group Position Paper," *Allergy* 71, no. 8 (2016): 1103–1134, https://doi.org/10.1111/all.12886.

5. A. Aranda, C. Mayorga, A. Ariza, et al., "In Vitro Evaluation of IgE-Mediated Hypersensitivity Reactions to Quinolones," *Allergy* 66, no. 2 (2011): 247–254.

6. K. Larimore, T. Nguyen, B. Badillo, et al., "Depletion of Interfering IgG and IgM Is Critical to Determine the Role of IgE in Pegvaliase-Associated Hypersensitivity," *Journal of Immunological Methods* 468 (2019): 20–28.

7. M. van Hage, C. Hamsten, and R. Valenta, "ImmunoCAP Assays: Pros and Cons in Allergology," *Journal of Allergy and Clinical Immunology* 140, no. 4 (2017): 974–977.

8. J. L. Paris, C. Monío, A. M. Pérez-Moreno, et al., "Influence of Pore Size in Protein G′-Grafted Mesoporous Silica Nanoparticles as a Serum Pretreatment System for in Vitro Allergy Diagnosis," *Advanced Healthcare Materials* 12, no. 15 (2023): e2203321.

9. A. Ariza, C. Mayorga, G. Bogas, et al., "Detection of Serum-Specific IgE by Fluoro-Enzyme Immunoassay for Diagnosing Type I Hypersensitivity Reactions to Penicillins," *International Journal of Molecular Sciences* 23, no. 13 (2022): 6992.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.