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Sedat ÇARKIT[a*]
Merve ÖZEL[b]
Bahadır ÖZ[c]
Mustafa GÖK[d]
Alper AKCAN[e]
Sevil DEMİRAY[f]
Hülya AKGÜN[g]
Gülden BAŞKOL[h]
Mustafa KARAAĞAÇ[i]
Gökmen ZARARSIZ[j]

[*a]Assist.Prof., Erciyes University, opdrsedatcarkit@gmail.com
[b]Assist.Prof., Erciyes University, ozelm381@gmail.com
[c]Prof.Dr., Erciyes University, drbahadir01@gmail.com
[d]Assoc.Prof., Erciyes University, mustafagok@erciyes.edu.tr
[e]Prof.Dr., Erciyes University, akcan2002@yahoo.com
[f]RA., Erciyes University, kocadag.se@gmail.com
[g]Prof.Dr., Erciyes University, hulyaakgun@yahoo.com
[h]Prof. Dr., Erciyes University, gbaskol@yahoo.com
[i]Assist.Prof., Erciyes University, drmustafakaraagac@gmail.com
[j]Prof.Dr., Erciyes University, Hematainer Biotechnology Inc. TEKNOPARK, gokmenzarasiz@erciyes.edu.tr

[*]Corresponding Author

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Prognostic Role of Gas6/sAXL Signaling Pathway in Predicting Treatment Resistance in Idiopathic Granulomatous Mastitis

Abstract

Idiopathic granulomatous mastitis is a rare chronic inflammatory breast disease with unclear pathogenesis and unpredictable therapeutic outcomes. Although the Gas6/sAXL signaling pathway plays an important role in immune regulation, its prognostic significance in idiopathic granulomatous mastitis remains unknown. This retrospective observational cohort study included 37 women with histopathologically confirmed idiopathic granulomatous mastitis, categorized as treatment-responsive (n=18) or treatment-resistant (n=19), and 17 age- and body mass index-matched healthy controls. Treatment resistance was defined as failure to achieve remission within 24 weeks of standard pharmacotherapy, or progression/persistence of abscess or sinus tract requiring surgical intervention after at least six weeks of treatment. Pre-treatment serum and formalin-fixed paraffin-embedded tissue levels of Gas6 and sAXL were measured using enzyme-linked immunosorbent assay. Receiver operating characteristic analysis evaluated discriminatory performance, while binary logistic regression identified independent predictors of treatment resistance. Serum Gas6 levels were significantly lower in both idiopathic granulomatous mastitis subgroups compared with controls (p<0.001). sAXL levels were significantly reduced in the resistant group; however, differences between resposive patients and controls were not significant after Dunn-Bonferroni correction. Receiver operating characteristic analysis demonstrated moderate discriminatory ability for Gas6 (AUC=0.747, p=0.003) and sAXL (AUC=0.727, p=0.006). A Gas6 cutoff value <1.9 pg/mL predicted treatment resistance with 74% sensitivity and 72% specificity. Multivariable analysis identified Gas6 <1.9 pg/mL (OR=5.18, 95% CI: 1.09-24.70, p=0.039) and abscess formation (OR=5.88, 95% CI: 1.14-33.3, p=0.035) as independent predictors of resistance, whereas sAXL lost significance. Reduced Gas6 levels are independently associated with treatment resistance in idiopathic granulomatous mastitis and, together with abscess formation, may serve as clinically useful markers for individualized therapeutic decision-making. Prospective multicenter studies are warranted to validate these findings.

Keywords: Biomarker, Gas6, idiopathic granulomatous mastitis, sAXL, treatment resistance.

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İdiyopatik Granülomatöz Mastitiste Tedavi Direncinin Öngörülmesinde Gas6/sAXL Sinyal Yolunun Prognostik Rolü

Öz

İdiyopatik granülomatöz mastit, etiyopatogenezi tam olarak bilinmeyen ve tedavi yanıtı değişkenlik gösteren nadir kronik inflamatuvar bir meme hastalığıdır. Gas6/sAXL sinyal yolunun

immün düzenlemede önemli rol oynadığı bilinmekle birlikte, idiyopatik granülomatöz mastit'deki prognostik değeri henüz araştırılmamıştır. Bu retrospektif gözlemsel kohort çalışmasına histopatolojik olarak doğrulanmış 37 idiyopatik granülomatöz mastit hastası dahil edildi ve hastalar tedaviye yanıtlı (n=18) ve dirençli (n=19) olarak sınıflandırıldı. Ayrıca yaş ve vücut kitle indeksi açısından benzer 17 sağlıklı kontrol değerlendirildi. Tedavi direnci, standart farmakoterapiye rağmen 24 hafta içinde remisyon sağlanamaması veya en az altı haftalık tedavi sonrasında cerrahi müdahale gerektiren apse ya da sinüs traktının ilerlemesi veya devam etmesi olarak tanımlandı. Tedavi öncesi serum ve formalinle fikse parafine gömülü doku örneklerinde Gas6 ve sAXL düzeyleri ELISA yöntemiyle ölçüldü. Ayırt edici performans analizi ile tedavi direncinin bağımsız belirleyicileri ise lojistik regresyon analizi ile değerlendirildi. Serum Gas6 düzeyleri her iki idiyopatik granülomatöz mastit grubunda kontrollere göre anlamlı derecede düşüktü (p<0.001). sAXL düzeyleri dirençli grupta anlamlı olarak düşük bulunurken, yanıtlı grup ile kontroller arasındaki fark Dunn-Bonferroni düzeltmesi sonrası anlamlılığını korumadı. Ayırt edici performans analizinde Gas6 için AUC 0.747 (p=0.003), sAXL için ise 0.727 (p=0.006) bulundu. Gas6 için <1.9 pg/mL eşik değeri tedavi direncini %74 duyarlılık ve %72 özgüllükle öngördü. Çok değişkenli analizde Gas6 <1.9 pg/mL ve apse varlığı tedavi direncinin bağımsız belirleyicileri olarak saptandı. Azalmış Gas6 düzeyleri idiyopatik granülomatöz mastit'de tedavi direnci ile bağımsız ilişkili olup, apse varlığıyla birlikte klinik karar süreçlerinde yararlı biyobelirteçler olabilir.

Anahtar kelimeler: Biyobelirteç, Gas6, idiyopatik granülomatöz mastitis, sAXL, tedavi direnci.

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Introduction

Idiopathic granulomatous mastitis (IGM) is an uncommon, non-infectious, chronic inflammatory condition of the breast that predominantly affects women of childbearing age, the majority of whom report a prior history of lactation.[1] Several etiological mechanisms have been proposed, including autoimmune dysregulation, hormonal influences, microbial triggers, and inherited susceptibilities such as alpha-1 antitrypsin deficiency.[2,3] The growing incidence of IGM reported in Mediterranean, Asian, and industrialized populations in recent years raises the possibility that environmental exposures or lifestyle-related factors may contribute to disease development.[4]

The clinical manifestations of IGM can closely resemble those of breast malignancy, encompassing palpable masses, skin erythema, abscess formation, draining fistulas, and regional lymphadenopathy.[3,4] The natural history of the disease is unpredictable, and therapeutic outcomes vary substantially across patients. Although corticosteroids, immunosuppressive agents, antimicrobials, and surgical procedures are all employed in management, the absence of validated prognostic biomarkers continues to hamper the establishment of evidence-based treatment protocols.[5,6]

The Gas6/sAXL axis has emerged as a significant regulator of inflammation and immune resolution. Growth arrest-specific protein 6 (Gas6) is a vitamin K-dependent secreted ligand belonging to the TAM receptor tyrosine kinase family (encompassing Tyro3, AXL, and MerTK); among these receptors, it binds AXL with the greatest affinity and mediates its principal downstream effects through this receptor.[7] Upon AXL engagement and activation, ectodomain shedding produces a soluble form designated sAXL, which competes with membrane-bound AXL for Gas6 binding and thereby attenuates receptor-mediated signal transduction.[8] Through this mechanism, the pathway curtails the elaboration of proinflammatory mediators including TNF-α, IL-6, and IFN-γ, supports efferocytotic clearance of apoptotic debris, and dampens excessive immune activation.[9] Experimental disruption of Gas6/AXL signaling has been shown to precipitate systemic autoimmunity and sustain chronic inflammatory states.[9]

Beyond systemic immune regulation, Gas6 is constitutively expressed in mammary epithelial cells, where it participates in macrophage polarization and tissue homeostatic remodeling.[10] In the setting of breast cancer, diminished Gas6 expression has been correlated with greater tumor invasiveness and adverse survival outcomes, whereas elevated levels are associated with more indolent tumor behavior and a more favorable prognosis.[11]

Given that IGM is pathologically defined by non-caseating granulomatous inflammation, is marked by relapsing disease flares, and displays heterogeneous responses to treatment, perturbations of Gas6/sAXL signaling could plausibly perpetuate the inflammatory milieu and underlie refractoriness to therapy.[11,12] Importantly, this putative role is mechanistically distinct from Gas6/sAXL simply acting as a passive correlate of inflammatory burden: because the pathway operates within the efferocytotic and pro-resolution arm of the immune response, rather than being consumed in proportion to inflammatory load as

occurs with conventional acute-phase reactants, a quantitative deficiency would be expected to impair the active resolution of granulomatous inflammation itself and could therefore contribute directly to treatment refractoriness rather than simply reflecting more severe or more active disease. To our knowledge, no prior investigation has measured Gas6 or sAXL in IGM patients or evaluated their utility as prognostic biomarkers. The present study therefore aimed to characterize the relationship between serum and tissue Gas6/sAXL levels and treatment outcomes in IGM, with the goal of identifying novel markers that may guide individualized clinical management.

Materials and Methods

Study Design and Ethics

This was a single-center, retrospective observational cohort investigation conducted among patients with IGM treated at a tertiary referral hospital between November 2019 and March 2021. An external group of healthy volunteers was recruited concurrently for comparative biomarker analyses. Histological diagnosis required the demonstration of non-caseating granulomatous inflammation-characterized by epithelioid histiocytes, Langhans-type multinucleated giant cells, lymphocytes, plasma cells, and eosinophils-in the absence of causative infectious organisms within the tissue specimen.

Ethical approval for the study protocol, including phlebotomy and retrieval of archival FFPE material, was granted by the Erciyes University Scientific Research Ethics Committee (Approval No: 2021/431). All participants provided written informed consent prior to enrollment, and the entire study was conducted in full adherence to the ethical principles of the Declaration of Helsinki.

Inclusion and Exclusion Criteria

Eligible participants were required to be at least 18 years of age, have a histopathologically confirmed diagnosis of IGM, and have complete documentation of clinical and therapeutic follow-up data.

Patients were excluded if they had any of the following: active infectious disease, concurrent malignancy, systemic granulomatous disorders (sarcoidosis, granulomatosis with polyangiitis, giant cell arteritis, polyarteritis nodosa), current pregnancy, personal history of pulmonary or extrapulmonary tuberculosis, presence of breast silicone implants, or a diagnosed genetic or immunological condition. When prominent suppurative or necrotic changes raised suspicion of secondary infectious mastitis, tissue sections were further examined with Ziehl-Neelsen, PAS, and GMS staining, and available microbiological culture data were reviewed. Any patient with confirmed bacterial, mycobacterial, or fungal pathogen involvement was subsequently excluded.

Patient Groups

Thirty-seven patients with IGM fulfilled the eligibility criteria. Of these, 18 constituted the treatment-responsive cohort (defined as sustained remission following medical management alone), while the remaining 19 formed the treatment-resistant cohort (defined as failure to achieve remission with medical therapy, necessitating surgical intervention). Treatment failure was operationally defined as the absence of clinically meaningful improvement or overt disease progression despite an adequate duration of first-line standard therapy, as evidenced by one or more of the following: unchanging or enlarging lesion dimensions, appearance of new inflammatory nodules, fistulas, or abscesses, persistence of local inflammatory signs, and/or escalation to more intensive treatment or surgery.

Seventeen healthy women matched for age and body mass index were enrolled as the control cohort. Control participants had no personal history of breast pathology, autoimmune conditions, or chronic inflammatory disease; normal breast histology was confirmed in all cases. Breast tissue specimens from the control group were procured from reduction mammoplasty samples that showed no inflammatory or neoplastic changes.

Group Comparability and Disease Severity

Pretreatment clinical and radiological parameters were systematically compared between the responsive and resistant groups to verify baseline equivalence. The two groups were well matched with respect to age, lesion dimensions, number of disease flares, leukocyte counts, and axillary lymph node involvement (all $p>0.05$). The sole statistically significant intergroup difference was a higher rate of abscess formation in the resistant cohort (84.2% vs. 38.9%, $p=0.004$). No participant in either group exhibited fistula formation or extramammary manifestations such as erythema nodosum or joint involvement. All patients received care within a uniform diagnostic and therapeutic framework at the same tertiary center.

Serum Sampling

Peripheral venous blood specimens were obtained from IGM patients immediately prior to the initiation of any anti-inflammatory or immunosuppressive treatment. Control group phlebotomy was carried out during a single dedicated visit following an identical collection protocol. Approximately 5 mL of

blood was drawn into gel-separator tubes; samples were permitted to clot for 30 minutes at ambient temperature and subsequently centrifuged for 10 minutes at 1,500-2,000 ×g. Resulting serum was partitioned into aliquots to prevent repeated freeze-thaw cycling, processed within 2 hours of collection, and preserved at −80 °C until batch analysis. All measurements were performed in duplicate or triplicate by personnel who were blinded to clinical outcome data.

Treatment Protocols and Surgical Interventions

The initial pharmacological regimen consisted of oral prednisolone administered at 0.5-1.0 mg/kg/day (target dose approximately 0.75 mg/kg/day) in accordance with our institutional guidelines. Dose tapering proceeded by reductions of 5-10 mg every one to two weeks until a maintenance dose of 5-10 mg/day was reached over 12-16 weeks; treatment was discontinued approximately 24 weeks after the achievement of remission. When the clinical response was insufficient or corticosteroid-related adverse events were unacceptable, a steroid-sparing immunosuppressant was added after four to six weeks of initial therapy: methotrexate 10-20 mg/week supplemented with folic acid 5 mg/week, or azathioprine 1.5-2.0 mg/kg/day in cases where methotrexate was contraindicated.

Patients were designated as "treatment-responsive" upon achieving complete clinical remission-defined by resolution of pain, induration, discharge, and fluctuance-accompanied by sonographic regression or disappearance of the inflammatory lesion, with no relapse during the tapering period. The "treatment-resistant" designation was applied under two circumstances: (i) failure to attain full remission by 24 weeks despite optimized pharmacotherapy (standard steroid regimen with or without a steroid-sparing agent), or (ii) progression or persistence of abscess, draining sinus tract, or newly emergent lesion requiring operative intervention at any point following at least six weeks of adequate treatment.

Surgical management was reserved for disease that proved refractory or developed complications, particularly when abscesses or sinus tracts were present. Operative procedures comprised ultrasound-guided percutaneous drainage and wide local excision of the inflammatory focus. Clinical reassessment incorporating physical examination and targeted ultrasonography was conducted every four to six weeks throughout the medical treatment phase.

Tissue Processing and ELISA

Diagnostic breast tissue biopsy specimens were available as formalin-fixed paraffin-embedded (FFPE) blocks generated during routine diagnostic pathology processing. For the purposes of this study, these FFPE blocks were used exclusively for protein extraction and ELISA-based quantification of Gas6 and sAXL. Ten-micrometre sections were cut from the FFPE blocks, deparaffinized, and subjected to mechanical homogenization. The resulting tissue homogenate suspensions were centrifuged, and the supernatants were stored at −80 °C until protein quantification. Total protein was extracted using RIPA buffer (Thermo Fisher Scientific, Cat. No: 89901) supplemented with a protease inhibitor cocktail (Roche, Cat. No: 11873580001).

Gas6 and sAXL concentrations in both serum and tissue extracts were measured using commercially available sandwich ELISA kits (BT LAB, Cat. Nos: E2029Hu and E7400Hu, respectively). All samples were assayed in triplicate, and laboratory personnel performing the analyses were blinded to the participants' clinical group assignments. No additional histopathological or immunohistochemical evaluation was performed on the FFPE specimens for the purposes of this study; therefore, no representative tissue images are presented.

Statistical Analysis

Distribution normality was assessed through graphical inspection (histograms and quantile-quantile plots) and the Shapiro-Wilk test; equality of variances was evaluated by Levene's test. Between-group differences in continuous outcomes were analyzed with the independent-samples t-test or the Mann-Whitney U test as appropriate, while categorical variables were compared using Pearson's chi-squared test or Fisher's exact test. Multi-group comparisons were performed with the Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc correction. ROC curves were generated to quantify the ability of baseline serum Gas6 and sAXL to discriminate treatment resistance, with AUCs reported alongside 95% confidence intervals. Optimal cut-off values were selected by minimizing the Euclidean distance from the ROC curve to the ideal point (0,1). Diagnostic performance metrics-sensitivity, specificity, PPV, and NPV-are presented with 95% CIs derived by the Clopper-Pearson method. Candidate variables meeting a liberal $p<0.10$ threshold and demonstrating clinical plausibility were entered into forward (Wald) stepwise multivariable logistic regression. Statistical significance was set at a two-tailed $p<0.05$. All statistical computations were performed using R statistical software (4.5.2 version, R Foundation for Statistical Computing, Vienna, Austria).

Results

The final analytic cohort comprised 37 IGM patients (Group R: n=18 treatment-responsive; Group NR: n=19 treatment-resistant) and 17 healthy controls. No statistically significant intergroup differences were identified for age, lesion size, gravidity, lactation history, number of inflammatory flares, white blood cell count, C-reactive protein (CRP), or duration of follow-up (all $p>0.05$). Notably, abscess formation was substantially more prevalent among treatment-resistant patients (84.2% vs. 38.9%, $p=0.004$) (Table 1).

Table 1. Demographic and clinical characteristics of patients

Variables	Resistant to treatment (Group NR, n=19)	Responsive to treatment (Group R, n=18)	*p*-value
Follow-up Period (month)	22.0 (13.0-36.0)	14.5 (9.0-17.0)	0.162
Lesion size (mm)	31.0 (22.0-50.0)	30.0 (23.0-40.0)	0.819
Gravidity	2 (1-2)	2 (0-2)	0.635
Number of flares	1 (1-2)	1 (1-2)	0.693
WBC ($\times 10^3/\mu L$)	10,100 (8,400-13,000)	9,650 (8,100-12,800)	0.796
CRP (mg/dL)	1.2 (0-3.8)	0.7 (0-2.1)	0.097
Age (years)	37.16±9.07	35.17±7.48	0.472
Undergoing Surgery	19 (100.0)	0 (0.0)	-*
Postmenopause	1 (5.3)	0 (0.0)	>0.99**
Axillary lymphadenopathy (LAP)	14 (73.7)	9 (50.0)	0.138
Abscess	16 (84.2)	7 (38.9)	0.004
Lactation	2 (10.5)	1 (5.6)	0.234
Smoking	1 (5.3)	2 (11.1)	0.604

Values are expressed as n (%), mean ± SD or median (1st-3rd quartiles). Significant p-values are shown in bold. *p*-values: independent-samples t-test or Mann-Whitney U test for continuous variables; Pearson's chi-square or Fisher's exact test for categorical variables (Fisher's exact test when any expected cell <5 or zero cell occurred). *Not tested (surgery is part of treatment-resistant management pathway). **Fisher's exact test.

Serum Gas6 concentrations were markedly lower in both IGM patient groups relative to healthy controls ($p<0.001$). Regarding sAXL, a significant reduction was observed in the treatment-resistant group compared with controls; however, the difference between the responsive group and controls did not attain significance following Dunn-Bonferroni post-hoc adjustment (overall Kruskal-Wallis $p=0.002$). When the two IGM subgroups were compared directly, the median Gas6 and sAXL levels were marginally lower in the resistant cohort, though this within-IGM difference did not reach statistical significance (Table 2).

Table 2. Serum Gas6 and sAXL levels in the groups

Protein concentrations	Control group (n=17)	Resistant to treatment (Group NR, n=19)	Responsive to treatment (Group R, n=18)	*p*-value
Gas6 (pg/mL)	a10.5 (8.7-20.6)	b1.6 (1.0-2.1)	b2.5 (1.6-4.2)	<0.001
sAXL (pg/mL)	a4.0 (2.5-6.4)	b1.9 (0.6-2.5)	a2.5 (1.7-6.6)	0.002

Matrix: Baseline serum samples (pre-treatment) analyzed using ELISA. Values are reported as median (1st-3rd quartiles). Overall *p*-values obtained from Kruskal-Wallis H test. Different superscripts (a, b) denote significant pairwise differences by Dunn-Bonferroni post-hoc testing at $p<0.05$; groups sharing a letter do not differ. Significant *p*-values are bold.

ROC analysis yielded AUC values of 0.747 for Gas6 ($p=0.003$) and 0.727 for sAXL ($p=0.006$). The Gas6 threshold of <1.9 pg/mL identified treatment-resistant patients with 74% sensitivity and 72% specificity, while the sAXL threshold of <2.3 pg/mL achieved 68% sensitivity and 61% specificity (Table 3, Figure 1). Applying these cut-offs, 14 of 19 resistant patients (73.7%) and 5 of 18 responsive patients (27.8%) had Gas6 levels below 1.9 pg/mL; for sAXL, the corresponding proportions were 13 of 19 resistant patients (68.4%) and 7 of 18 responsive patients (38.9%) with sAXL levels below 2.3 pg/mL.

Table 3. ROC curve and diagnostic statistics of serum Gas6 and sAXL levels for discriminating treatment resistance

Marker (serum cut-off)	AUC (95% CI)	*p*-value	SEN (95% CI)	SPE (95% CI)	PPV (95% CI)	NPV (95% CI)
Gas6 <1.9 pg/mL	0.75 (0.67-0.82)	0.003	0.74 (0.49-0.91)	0.72 (0.47-0.90)	0.74 (0.49-0.91)	0.72 (0.47-0.90)
sAXL <2.3 pg/mL	0.73 (0.63-0.86)	0.006	0.68 (0.43-0.87)	0.61 (0.36-0.83)	0.65 (0.41-0.85)	0.65 (0.38-0.86)

ROC: receiver operating characteristics; AUC: area under the curve; SEN: sensitivity; SPE: specificity; PPV: positive predictive value; NPV: negative predictive value; CI: confidence interval. CI for PPV and NPV calculated using Clopper-Pearson method. Significant *p*-values are bold.

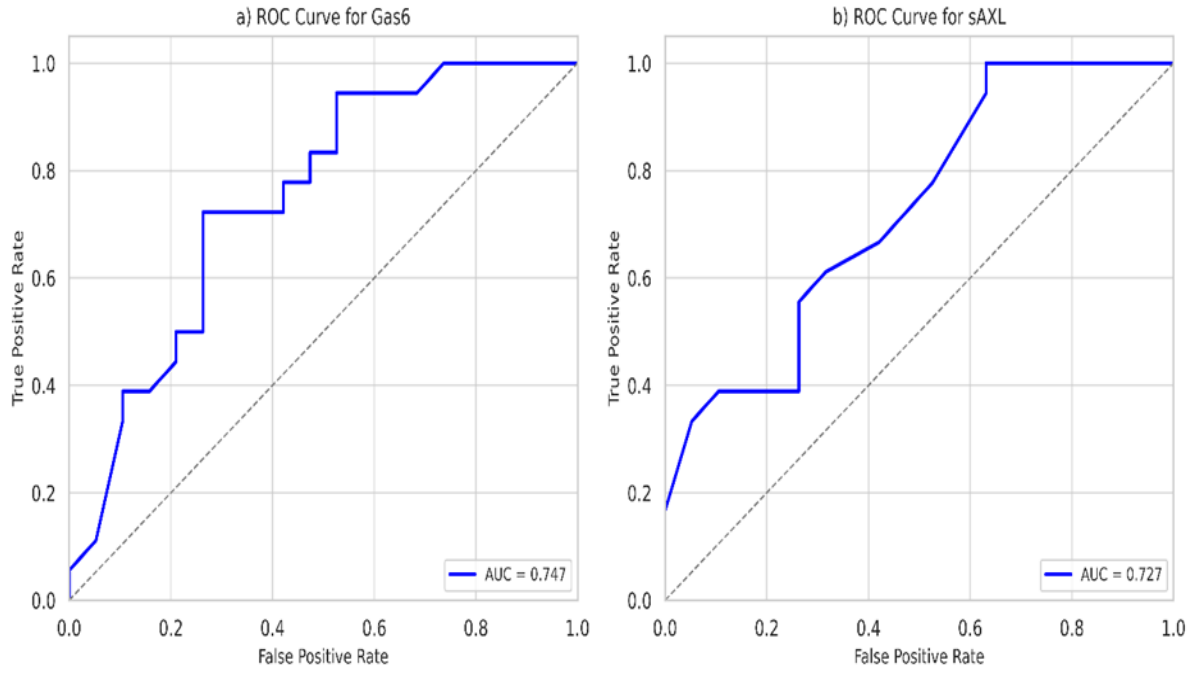

Figure 1. Receiver operating characteristic (ROC) curves showing the discriminatory performance of serum Gas6 (A) and sAXL (B) for predicting treatment response in patients with IGM. Gas6 demonstrated an AUC of 0.747, while sAXL demonstrated an AUC of 0.727, indicating moderate discriminatory performance. The diagonal dashed line represents discrimination equivalent to chance (AUC = 0.50).

Protein quantification in FFPE tissue specimens yielded findings that mirrored the serum data: both Gas6 and sAXL were diminished across IGM subgroups relative to controls, with somewhat lower median values in the resistant group; the difference between the two IGM subgroups remained non-significant (Figure 2).

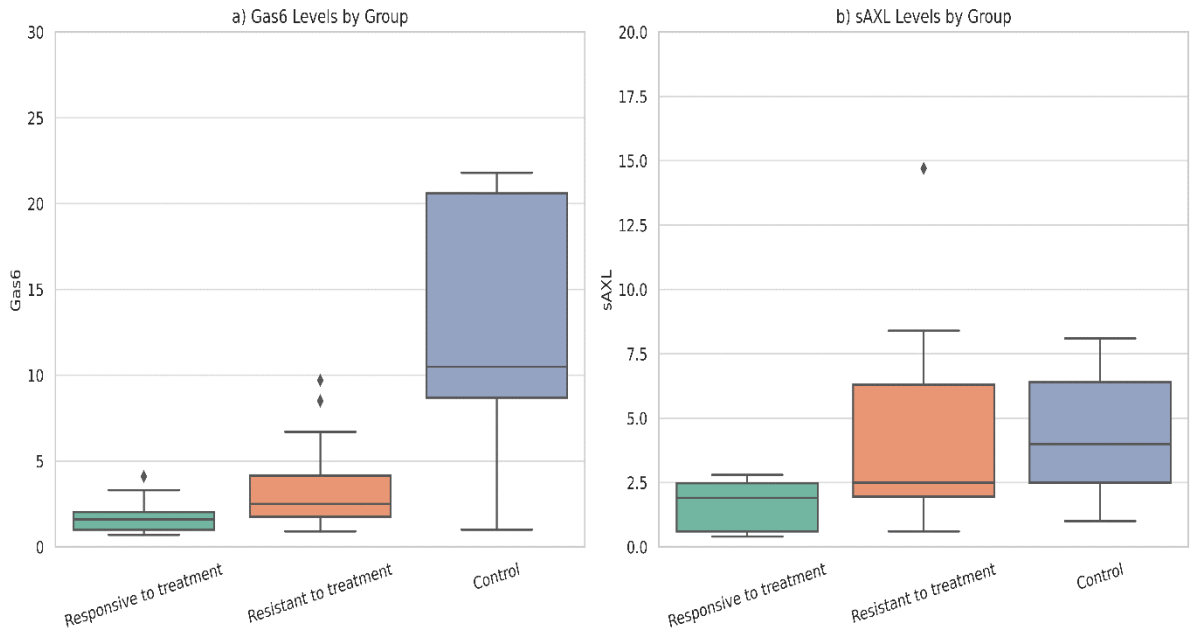

Figure 2. Box-and-whisker plots depicting serum Gas6 and sAXL concentrations stratified by study group. Both biomarkers were significantly reduced in IGM patients compared with healthy controls. Gas6 reached its lowest values in treatment- resistant patients, was intermediate in the responsive cohort, and highest in controls. The sAXL pattern differed: a significant decline was observed exclusively in the resistant group, with no statistically significant difference detected between treatment- responsive patients and healthy controls.

Binary logistic regression identified two independent predictors of treatment resistance: abscess formation (OR=5.88, 95% CI: 1.14-33.3, p=0.035) and Gas6 below the 1.9 pg/mL threshold (OR=5.18, 95% CI: 1.09-24.70, p=0.039). Although sAXL exhibited a borderline association at the univariate stage, it failed to emerge as an independent contributor in the multivariable model (Table 4).

Table 4. Logistic regression analysis of factors that can be used to predict treatment resistance

Variable	Univariate OR (95% CI)	Univariate *p*-value	Multivariate OR (95% CI)	Multivariate *p*-value
Age (>37 years)	1.39 (0.38-5.07)	0.619	-	-
Lesion size (>30 mm)	0.72 (0.20-2.63)	0.619	-	-
Axillary LAP (present)	2.80 (0.71-11.10)	0.143	-	-
Abscess (present)	8.33 (1.75-33.3)	0.007	5.88 (1.14-33.3)	0.035
Gas6 (<1.9 pg/mL)	7.28 (1.71-31.08)	0.007	5.18 (1.09-24.70)	0.039
sAXL (<2.3 pg/mL)	3.41 (0.88-13.19)	0.076	-	-

OR: Odds ratio; CI: Confidence interval. Significant p-values are bold. Age, lesion size, and axillary LAP (each p>0.10) were not entered into the multivariable model. sAXL met the p<0.10 screening threshold (p=0.076) but was not retained in the final stepwise model. The final model showed adequate goodness-of-fit (Hosmer-Lemeshow test, p>0.05), explained approximately 38% of the variance (Nagelkerke R^2 = 0.38), and correctly classified 79.4% of patients.

Discussion

IGM is a chronic benign inflammatory breast condition whose clinical and radiological features can closely mimic malignancy, resulting in delayed diagnoses and non-uniform management strategies.[13] Even after more than four decades of reported cases, the underlying etiopathogenesis remains incompletely understood, with autoimmune, hormonal, infectious, and genetic contributors all implicated, perpetuating a largely empirical and inconsistent approach to treatment.[2,4] We chose to investigate the Gas6/sAXL signaling axis in view of its well-characterized capacity to suppress inflammation, facilitate efferocytosis, and uphold immune homeostasis.[14-17] Our principal findings were that Gas6 concentrations were markedly reduced in both IGM subgroups relative to controls, while sAXL was significantly lower specifically in the resistant cohort, with the responsive-versus-control comparison failing to achieve post-hoc significance.

A methodologically notable feature of this work is the quantification of Gas6 and sAXL in archival FFPE breast tissue using ELISA-an approach that enabled retrospective analysis of banked specimens and exemplifies translational application of biomarker research. The tissue-level findings were concordant with those derived from serum, reinforcing the association between attenuated Gas6/sAXL signaling and unfavorable therapeutic outcomes.

Logistic regression analysis demonstrated that a serum Gas6 concentration below 1·9 pg/mL independently predicted resistance to standard treatment. At this cut-off, Gas6 achieved 74% sensitivity and 72% specificity in discriminating resistant from responsive patients; the corresponding sAXL threshold of <2.3 pg/mL yielded somewhat lower discrimination (68% sensitivity, 61% specificity). These data suggest that Gas6 may be a more informative biomarker candidate than sAXL for IGM risk stratification, although this comparison requires confirmation in larger, independent cohorts. Although sAXL showed a univariate association, it was not retained as an independent predictor in multivariable modeling, suggesting that its additive value over Gas6 is limited.

The present results align with established mechanistic data demonstrating that Gas6/AXL signaling attenuates NF-κB-driven inflammatory cascades, suppresses proinflammatory cytokine output including TNF-α and IL-6, and skews macrophages toward anti-inflammatory phenotypes.[18-20] Given the central contribution of activated macrophages to granuloma assembly in IGM, these pathways are especially pertinent. Prior investigations have documented elevated circulating concentrations of IL-6, IL-8, IL-10, IL-17, and IL-33 in IGM patients.[21, 22] The depressed Gas6 and sAXL levels we observed may reflect an impaired capacity to counter-regulate these proinflammatory mediators, thereby promoting treatment refractoriness. The inclusion of healthy controls adds important reference data: substantially higher biomarker levels in controls suggest that Gas6 and sAXL may serve as markers not merely of disease presence but also of disease severity and the likelihood of a favorable therapeutic response. Notably, conventional indices of inflammatory burden - CRP, leukocyte count, lesion size, and flare frequency - did not differ significantly between the resistant and responsive subgroups (Table 1), whereas Gas6 did and remained an independent predictor of resistance on multivariable analysis; this dissociation argues against Gas6 acting merely as a proxy for overall inflammatory severity and instead supports a more specific mechanistic contribution to treatment refractoriness.

These findings are hypothesis-generating: patients with Gas6 concentrations below the ROC-defined cut-off may warrant closer surveillance or earlier consideration of surgical or combined treatment approaches. However, given the retrospective design, modest sample size, and single-center setting of this study, this potential application should not yet guide clinical decision-making; prospective, multicenter studies with external validation are required before Gas6-based risk stratification could be considered for routine practice.

In the present cohort, abscess formation also emerged as a robust independent predictor of treatment resistance (OR≈5.9). Abscesses were present in 84.2% of resistant versus 38.9% of responsive patients, an association that persisted in both univariate and multivariable models. In line with observations by Lermi et al.,[23] abscess development appears to signal not only advanced disease severity but also an inherently higher probability of requiring more aggressive intervention.

The reported associations between diminished Gas6/sAXL concentrations and systemic autoimmune conditions such as systemic lupus erythematosus and Behçet's disease[24-28] raise the possibility that IGM shares underlying immunopathological mechanisms with these immune dysregulation syndromes. The Gas6/sAXL axis may therefore represent a convergent therapeutic target amenable to intervention across a spectrum of chronic inflammatory conditions.

Several methodological limitations warrant consideration. The modest cohort size restricts statistical power, particularly for subgroup analyses; notably, the power estimate for sAXL was only moderate (60.6%), meaning that certain associations may have been insufficiently powered to detect. The retrospective, single-center design precludes causal inference regarding the role of Gas6/sAXL dysregulation in IGM pathogenesis. No serial biomarker measurements were available to characterize temporal fluctuations in Gas6 or sAXL over the course of disease. Finally, the findings originate from a single referral center, limiting their broader generalizability pending external validation.

Conclusion

Although both Gas6 and sAXL were reduced in patients with IGM, only Gas6 independently predicted treatment resistance in multivariable analysis. These results support further investigation of serum Gas6 measurement, alongside the clinical finding of abscess formation, as candidate components of a composite risk-stratification framework that could, pending validation, help inform earlier selection

between conservative and surgical or combined treatment approaches. Multicenter prospective validation studies are necessary to establish the clinical utility of these biomarkers before any such framework could be applied in routine practice.

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Declarations:
1. Originality Statement: This study is original and has not been published previously.
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3. Ethics Committee Approval: Ethics committee approval for this study was obtained from the Erciyes University Scientific Research Ethics Committee (Approval No: 2021/431).
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5. Conflict of Interest: The authors declare that there are no conflicts of interest.
6. Generative Artificial Intelligence Statement: Generative artificial intelligence tools, namely Claude Sonnet 5 (Anthropic) was used for improving language and readability.
7. Sustainable Development Goals: This work is related to the following United Nations Sustainable Development Goals

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