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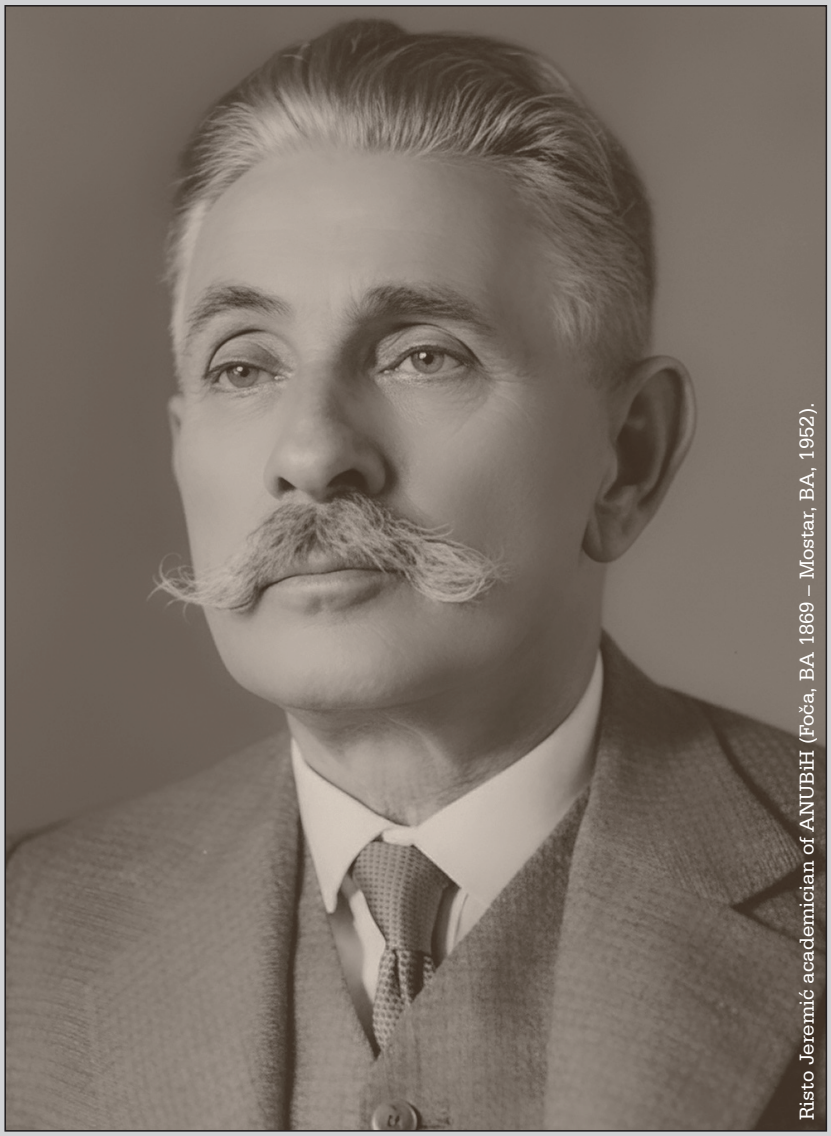

Risto Jeremić academician of ANUBiH (Foča, BA 1869 – Mostar, BA, 1952).

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COVER PHOTO PICTURE

Risto Jeremić (Foča, 18 May 1869 – Mostar, 17 September 1952) graduated from the Faculty of Medicine in Graz in 1898. He worked as a surgeon at the National Hospital in Sarajevo and, for a short time, served as its director. He later worked as a surgeon in Tuzla and as the head of the medical department of the State Railways Directorate in Subotica. He participated as a volunteer doctor in the Balkan Wars. He was the author of more than 100 papers in the fields of public health and medicine in Yugoslavia. He was an honorary doctor of the University of Belgrade and one of the first members of the Scientific Society of Bosnia and Herzegovina.

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PhD Theses Defended in Croatia (1992-2023): A Retrospective Analysis of Trends, Institutional Contributions, and Data Collection Challenges

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Abstract

Objective. This study analyzed PhD theses defended in Croatia between 1992 and 2023, with the aim of examining national trends, institutional contributions, disciplinary patterns, and data-related challenges. **Methods.** This retrospective time-trend study utilized the administrative data obtained from the Croatian Bureau of Statistics. Data on the number of defended PhD theses were collected by year, university, and school/department. Linear regression models were applied to assess temporal trends at both the national and institutional levels. **Results.** A total of 17,578 PhD theses were defended in Croatia between 1992 and 2023. The national output increased substantially, reaching a peak of 1,338 theses in 2012, followed by a subsequent decline and a gradual recovery. The University of Zagreb accounted for 74.8% of all defended theses, followed by the Universities of Osijek, Rijeka, and Split. Across institutions, the medical, economic, and engineering faculties were the most productive. Linear regression analyses demonstrated statistically significant upward trends at both the national level and across all major public universities. Collectively, medical schools produced 18% of all theses, with newer institutions, particularly those in Split and Osijek, exhibiting later but consistent growth. However, notable data inconsistencies were observed, including non-standardized institutional nomenclature, variable data granularity, and discrepancies among official reports. **Conclusion.** Croatia's PhD output expanded markedly after 2000, reflecting the maturation and expansion of its higher education system. Regional universities and medical schools substantially increased their contributions, indicating national academic growth. Sustained institutional support will be essential to sustain progress and foster disciplinary development.

Key Words: Academic Dissertations as Topic ▪ Higher Education ▪ Universities ▪ Croatia.

Introduction

PhD (doctoral) education plays a pivotal role in advancing a nation's research capacity, innovation potential, and overall socio-economic development (1, 2). Croatia gained independence in 1991, and in the decades since, its investment in research and development has steadily increased – reaching 1.1 billion euros in 2023. This corresponded to 1.39% of GDP, with higher education institutions accounting for 28% of this expenditure (3). Despite this progress, limited research has examined the structure and outcomes of PhD programs in Croatia.

In 2023, Vrdoljak published an analysis of trends in PhD graduations in Croatia and other European Union (EU) member states (4). Using data from the Statistical Database of the European Commission (Eurostat) for 35 European countries during the period 2013–2019, the study found that the highest numbers of doctoral graduates per 1,000 inhabitants were recorded in the most economically and socially developed Western European nations. For Croatia, this analysis revealed regional disparities and gender differences in PhD attainment, emphasizing the need for detailed studies to inform equitable educational policies and lifelong learning strategies (4).

Comprehensive longitudinal analyses of doctoral output in Croatia remain scarce, particularly

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those addressing institutional contributions, disciplinary patterns, and data quality issues. Addressing these gaps is crucial to improving doctoral education and strengthening the national research ecosystem. The scarcity of studies on this topic is likely due to difficulties in obtaining data on PhD studies and their outputs in Croatia. In 2024, we reported a case study describing our attempt to compile a complete list and full texts of PhD theses defended at Croatian medical schools between 1992 and 2021 (5). Despite extensive communication with national institutions, universities, and libraries, no single, complete database was available. By consolidating data from four different sources, we identified 2,955 theses, although the availability of full texts online was limited. Only 22% of the PhD theses in the sample were accessible through the national repository, while Zagreb and Split had only partial institutional access to the full text of the theses. The National and University Library held 90% of the targeted PhD theses in print form. This case study underscored substantial barriers to thesis accessibility and highlighted the need for greater transparency to support scientometric research and evidence-based science policy (5).

This study aimed to address this knowledge gap by providing a comprehensive analysis of PhD theses defended in Croatia between 1992 and 2023, focusing on national trends and institution-level contributions.

Methods

Study Design and Setting

This retrospective time-trend analysis utilized national administrative data to examine PhD theses defended across all academic disciplines in Croatia between January 1, 1992, and December 31, 2023. The starting year for the data collection was chosen because Croatia declared its independence on June 25, 1991, when the Croatian Parliament adopted the *Constitutional Decision on the Establishment of the Sovereign and Independent Republic of Croatia* (6).

Reporting

The study was reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for observational research, where applicable, with a focus on aggregated institutional and temporal trends (7).

Data Sources

Data were obtained from the Croatian Bureau of Statistics (Croatian: *Državni zavod za statistiku*, DZS). For the period 2004-2023, data were retrieved from the annual reports published on the DZS website. These reports, available in PDF format, contain both textual and tabular information. Data were extracted from these files.

As of May 2025, detailed information on the number of PhD theses defended at individual institutions in 2024 had not yet been published. For the period 1993-2003, the DZS did not have publicly available reports. These data were collected by visiting the DZS office in Zagreb, where the first author (LP) was granted access to printed statistical yearbooks. Tables containing data on the number of defended PhD theses were photographed and subsequently transcribed manually into a spreadsheet for analysis.

Data Extracted

The extracted variables included: the number of PhD theses defended annually from 1992 to 2023, the name of the university, and the name of the corresponding school or faculty.

Variables Analyzed

The primary outcome was the annual number of defended PhD theses, analyzed at the national, university, and school/department levels. Temporal trends in doctoral production between 1992 and 2023 were examined, and a subgroup analysis was performed for medical schools. Additionally, challenges encountered in accessing and analyzing the data were documented.

Ethics

This study utilized fully anonymized, aggregate administrative data that contained no personal identifiers. Therefore, ethical approval was not applicable.

Statistical Analysis

Descriptive statistics were presented as frequencies and percentages. The analyses were based on annual counts of defended doctoral theses and were not adjusted for the number of active doctoral programs. Namely, longitudinal program-level data by institution were not available in the national reports used as the data source. Consequently, the findings should be interpreted as absolute output measures rather than productivity per program. Temporal trends in the number of defended PhD theses were assessed using a series of linear regression analyses. Ordinary least squares (OLS) regression was applied to model annual changes in thesis counts. This analysis was conducted for descriptive purposes only; no Poisson, negative binomial, or other count models were fitted and no sensitivity analyses comparing model families were performed. Accordingly, the estimated coefficients (and any reported P-values) should be interpreted as descriptive trend indicators rather than confirmatory evidence.

The dependent variable was the annual number of defended PhD theses, and the independent variable was the calendar year (1992–2023). A separate linear regression model was developed for each institution to estimate the direction and magnitude of trends over time. Institutions with fewer than ten annual data points were excluded from regression analyses to ensure adequate statistical power and model stability.

The slope coefficient of each regression line indicated the estimated average annual change in the number of defenses. The statistical significance of each slope was evaluated using a t-test, with a significance threshold of $P<0.05$. Model fit was assessed using the coefficient of determination (R^2), indicating the proportion of variance in thesis counts explained by year. Trends were categorized as "increasing," "decreasing," or "stagnating" based on the direction and statistical significance of the slope – significantly positive slopes indicated increasing trends, significantly negative slopes indicated decreasing trends, and non-significant slopes were classified as stagnating. All statistical analyses were conducted using Microsoft Excel 2021 (Microsoft Corporation, Redmond, WA, USA).

Raw Data Availability

The raw data collected for this study are publicly available on the Open Science Framework at https://osf.io/y5wu8/.

Results

A total of 17,578 PhD theses were defended in Croatia between 1992 and 2023. The annual number of theses defended increased substantially from 234 in 1992 to a peak of 1338 in 2012, followed by a sharp decline and a substantial period of gradual recovery (Figure 1A, Supplementary Table 1).

PhD Output per University

Comparative analysis per university revealed that the majority of theses (74.8%) were defended at the University of Zagreb, followed by the University of Osijek (8.0%), the University of Rijeka (7.5%), and the University of Split (6.6%). Other higher education institutions contributed smaller proportions, each accounting for less than 3% of the total number of defended theses (Table 1).

Temporal Trends and Linear Regression Analysis

Table 2 summarizes the temporal trends in the number of defended PhD theses in Croatia from 1992 to 2023, based on linear regression analyses conducted for the national dataset and for selected public universities with more than ten annual data points. The five universities included in this analysis were the University of Osijek, the University

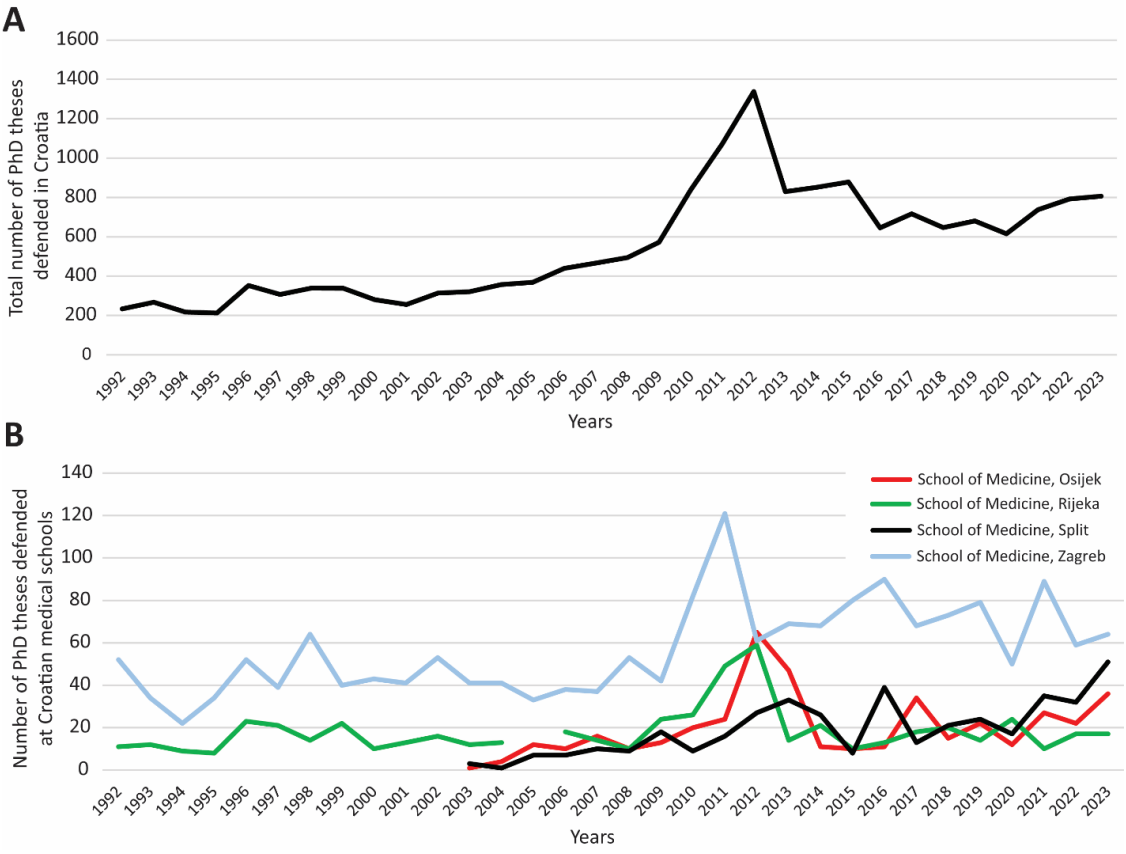

Figure 1. Temporal trends in the number of defended PhD theses in Croatia (1992–2023).
Figure 1A shows the total number of PhD theses defended annually across all Croatian higher education institutions over the 32 analyzed years. Figure 1B presents the cumulative number of defended PhD theses in medical schools in Osijek, Rijeka, Split and Zagreb during the same time frame.

Table 1. PhD Theses Defended in Croatia per University (1992-2023)

University	N (%)
Catholic University of Croatia	9 (0.05)
University North	15 (0.09)
University of Osijek	1405 (8.0)
University of Pula	63 (0.4)
University of Rijeka	1319 (7.5)
University of Slavonski Brod	8 (0.05)
University of Split	1156 (6.6)
University of Zadar	401 (2.3)
University of Zagreb	13152 (74.8)
Scientific legal entities*	50 (0.3)
Total	17578 (100)

*From 1993-1995, this common name was used for doctorates defended at the Institute Ruđer Bošković and the Institute for Medical Research and Occupational Health.

of Rijeka, the University of Split, the University of Zadar, and the University of Zagreb. Institutions excluded from this analysis due to insufficient data were the Catholic University of Croatia, University North, University of Pula, University of Slavonski Brod, and scientific legal entities.

At the national level, there was a statistically significant upward trend in the number of defended PhD theses, with an estimated average annual increase of 22.63 theses per year (R^2=0.574, t=6.36, P<0.001). This result indicates a moderately strong association between time and doctoral output, suggesting a sustained expansion in doctoral education during the study period (Table 2).

At the institutional level, all analyzed universities demonstrated statistically significant positive trends, although with varying slopes and model fits.

Table 2. Temporal Trends in the Number of Defended PhD Theses in Croatia by Institution (1992–2023); Linear Regression Analysis*

University	Slope	Intercept	R2	t-stat; P-value	Trend
Croatia – all PhD theses	+22.63	-44,885.83	0.574	6.36; P<0.001	Increasing
University of Osijek	+3.30	-6577.24	0.537	5.90; P<0.001	Increasing
University of Rijeka	+1.74	-3451.67	0.486	5.33; P<0.001	Increasing
University of Split	+2.94	-5770.12	0.499	5.50; P<0.001	Increasing
University of Zadar	+0.64	-1188.79	0.310	3.54; P<0.010	Increasing
University of Zagreb	+7.29	-14,318.60	0.185	2.75; P=0.010	Increasing

*The analysis made only for universities with more than 10 data points.

The University of Zagreb, the largest Croatian university, showed a statistically significant increase of 7.29 theses per year (t=2.75, P=0.010), albeit with a relatively low explanatory power (R^2=0.185), indicating considerable year-to-year variability not fully explained by time alone (Table 2).

Dominant Schools/Departments within Universities

For some universities, the DZS did not report data disaggregated by school/department. Where such data were available, the most successful schools/departments are shown in Table 3. At the University of Osijek, the schools of medicine, economics, and agriculture/agrobiotechnical sciences collectively accounted for 64% of all defended PhD theses. Similarly, at the University of Rijeka, the School of Medicine, the School of Economics, and the Faculty of Engineering represented 66% of all defended theses. At the University of Split, the School of Medicine, the Faculty of Electrical Engineering, Mechanical Engineering, and Naval Architecture, and the School of Economics, accounted for 67% of all doctorates. At the University of Zagreb, the three highest ranking institutions, based on the number of defended PhD theses, were the Faculty of Science, the Faculty of Humanities and Social

Table 3. Highest Ranking Schools/departments Based on Defended PhD Theses

University/School	N (%)
University of Osijek (N=1405)	
School of Medicine	422 (30)
School of Economics	286 (20)
School of Agriculture/Agrobiotechnical Sciences	190 (14)
University of Rijeka (N=1319)	
School of Medicine	562 (43)
School of Economics	161 (12)
Faculty of Engineering	148 (11)
University of Split (N=1156)	
School of Medicine	406 (35)
Faculty of Electrical Engineering, Mechanical Engineering and Naval Architecture	196 (17)
School of Economics	179 (15)
University of Zagreb (N=13152)	
Faculty of Science	2512 (19)
Faculty of Humanities and Social Sciences	2415 (18)
School of Medicine	1812 (14)

Sciences, and the School of Medicine; they accounted for 51% of defended PhD theses at the University of Zagreb (Table 3).

The Role of Medical Schools in PhD Output

Across the analyzed period, four medical schools, those of Zagreb, Rijeka, Split and Osijek, collectively produced 3202 PhD theses, representing 18% of the total national output. The older medical schools in Zagreb and Rijeka awarded doctorates throughout the entire study period, whereas the newer schools in Osijek and Split began awarding doctorates in 2003 (Figure 1B; Supplementary Table 2).

Data Quality, Consistency, and Reporting Anomalies

The analysis revealed several data-related challenges, including inconsistent reporting of institutional names and structures, variations in the granularity of available data (university-level vs. department-level), and ambiguity regarding the inclusion of doctoral degrees in the arts. Additional issues included nonspecific or duplicative entries and discrepancies between preliminary and finalized datasets. Manual extraction of data from older printed sources, the absence of standardized data organization (e.g., alphabetical ordering before 2019), and occasional typographical or reporting errors further complicated longitudinal comparisons and hindered the accuracy and reproducibility of analyses (Table 4).

Discussion

This comprehensive analysis of doctoral output in Croatia between 1992 and 2023 provides valuable insights into the evolution, growth, and institutional contributions to PhD education over three decades. The observed patterns reflect both the broader structural transformation of Croatia's higher education system and the distinct developmental trajectories of individual institutions.

Following Croatia's declaration of independence in 1991, the national trend reveals a pronounced expansion in doctoral output. In the early 1990s, the number of defended doctoral theses was very low—a finding attributable to

Table 4. Overview of Data Quality Issues in PhD Thesis Records Reported by the Croatian Bureau of Statistics (DZS)

Challenge	Example
Changes in institution names and structures.	The School of Humanities and Social Sciences in Zadar was once part of the University of Split, but later became part of a new university.
Inconsistent naming conventions in published data.	Most data by DZS were categorized by university names, while some were labeled geographically (e.g., "Area Split").
Inconsistencies in the granularity of data.	The Faculty of Humanities and Social Sciences from Zagreb had data displayed sometimes as a whole and other times as individual departments.
Varying levels of aggregation	Some data were reported at the department level, others at the university level, and in some cases, both presentations existed throughout the analyzed period.
Possible inclusion of doctorates in the arts, which may not be distinguished from science doctorates.	Some institutions, such as Music Academy, Academy of Fine Arts, are also on the DZS lists.
Nonspecific entries	Entries such as "University of Osijek" are listed as both a university and a school/department in some years.
Manual data extraction challenges	Data before 2004 was only available in printed monographs and had to be manually photographed and extracted.
Lack of alphabetical sorting in data tables until 2019, reducing standardization.	In data tables until 2019, the institutions were not listed alphabetically.
A discrepancy in reported figures	For the year 2018, one DZS report listed 647 defended PhDs, while another listed 628. Upon contacting DZS, the correct number (647) was confirmed; the lower number was from a preliminary report.

the Croatian War of Independence (1991-1995). The war had a profound adverse impact on the national education system, reducing enrollment in higher education, disrupting academic activities, and mobilizing a portion of the student population into active military service (8-10).

Subsequent years saw a steady increase in doctoral defenses, reflecting the gradual recovery and development of the higher education and research sectors. This growth culminated in a peak of 1,338 defended doctorates in 2012, followed by a marked decline. The 2012 peak likely resulted from a transitional provision in the 2003 *Croatian Act on Scientific Activity and Higher Education*, which abolished the pre-Bologna master of science (Croatian: *magisterij znanosti*) degree. The act allowed holders and enrollees of that degree program to obtain a PhD by defending a dissertation without enrolling in a formal doctoral program until 2011, with the final deadline extended to 2012 (11-13).

Regression analyses confirmed a statistically significant national upward trend, with an estimated average annual increase of 22.63 defended theses (slope = +22.63; $P<0.001$). This finding demonstrates a sustained expansion of doctoral training in Croatia, driven by higher education reforms, and the implementation of the Bologna Process (14, 15).

Reliable and internationally comparable data on the number of newly awarded doctorates remain limited. However, the global trend toward the expansion of doctoral education mirrors the Croatian experience. Among memers states of the Organisation for Economic Co-operation and Development (OECD), available data indicate that the number of doctoral degrees nearly doubled between the mid-1990s and 2017 (16). Furthermore, doctoral attainment in OECD countries increased by 25% between 2014 and 2019 (17), underscoring a worldwide emphasis on the expansion of doctoral training.

Dominance of the University of Zagreb

The University of Zagreb accounted for 75% of all defended PhD theses during the study period. While this dominance is unsurprising given the university's size, disciplinary breadth, and long-standing tradition of doctoral education, it raises important considerations regarding the concentration of doctoral training and the potential risks associated with over-centralization (18). The University of Zagreb also exhibited a statistically significant, though comparatively less stable, upward trend in doctoral output ($R^2=0.185$). This weaker model fit likely reflects internal institutional diversity and varying research capacities among its faculties.

Other public universities with at least a decade of recorded data, specifically Osijek, Rijeka, Split, and Zadar, also demonstrated statistically significant positive trends, albeit with smaller total outputs. The Universities of Osijek, Rijeka, and Split showed moderate-to-strong model fits (R^2 between 0.49 and 0.54), indicating more consistent year-on-year increases in doctoral production. These results suggest successful institutional consolidation and gradual expansion of doctoral education outside the capital, consistent with the decentralization goals of the European Higher Education Area (EHEA) (18).

Regional Diversification and New Universities

Beyond Zagreb, regional universities, particularly those in Osijek and Split, have shown substantial growth in the number of schools/departments awarding PhD degrees over the analyzed period. This expansion underscores a broader national effort to diversify and strengthen research capacity across Croatia, reflecting both regional development strategies and the increasing maturity of academic institutions.

The emergence of PhD programs in newer universities, including the University of Zadar, the Catholic University of Croatia, University North, and the University of Slavonski Brod, further indicates an ongoing diversification within the Croatian higher education landscape. Although their overall contributions remain modest and relatively recent, these institutions represent important steps toward building research capacity and promoting balanced academic development across different regions of the country.

Disciplinary Concentration in Medicine, Economics, and Engineering

At the Universities of Osijek, Rijeka, and Split, three dominant schools accounted for approximately two-thirds of all defended PhD theses, suggesting a strong concentration of doctoral education within a limited number of disciplines. The consistent prominence of medical, economic, and engineering faculties reflects clear disciplinary trends and institutional priorities. These findings are consistent with prior research showing that STEM and biomedical fields tend to receive greater institutional investment and attract larger numbers of doctoral candidates due to more defined academic and professional career pathways (6).

At the University of Zagreb, by far the largest contributor to the national doctoral output, the highest-ranking three schools collectively accounted for just over half (51%) of all defended theses. This indicates comparatively greater disciplinary diversity. Nevertheless, these results reveal persistent imbalances across fields and may reflect broader structural factors, including disparities in research funding, institutional strategies, and labor market demand. Such disciplinary concentration warrants further investigation into the alignment of national research priorities with doctoral education policy and institutional support for underrepresented academic areas.

Medical Schools as Key Drivers of Doctoral Output in Croatia

Medical schools at the universities of Zagreb, Rijeka, Split, and Osijek were among the most productive institutions in Croatia in terms of doctoral output. The strong performance, particularly of the Universities of Zagreb and Rijeka, can be attributed to their earlier establishment and long-standing academic traditions. The University of Zagreb School of Medicine was founded in 1917 (19), and the University of Rijeka School of Medicine in 1955 (20). Their longer institutional histories have facilitated the development of robust research infrastructure, extensive international collaborations, and close integration with research-intensive hospital systems.

By contrast, the delayed onset of doctoral activity at the newer medical schools in Split and Osijek was expected, given their later founding dates. The University of Split School of Medicine was founded in 1997 (21), and the University of Osijek School of Medicine in 1998 (22). Despite their recent origins, both institutions rapidly implemented doctoral programs, with the first theses defended in 2003. The upward trend in defended PhD theses observed at both schools indicates the successful establishment and progressive development of their doctoral training programs.

Although the overall number of medical doctoral theses in Croatia is relatively high, only a limited number of studies have examined the quality, productivity, and structure of medical PhD education. In 2003, Frković et al. published an analysis of publication output derived from doctoral and master's theses defended at the medical schools in Rijeka and Osijek. They found that only a minority of theses resulted in published scientific articles and emphasized the need for stronger institutional support to facilitate dissemination of doctoral research (23).

In 2017, we reported an interventional study aimed at improving completion rates among medical PhD students (24). Conducted at the University of Split School of Medicine, this study demonstrated that implementing stricter admission criteria, regulatory measures, and curriculum reforms in a newly established biomedical PhD program significantly increased completion rates and reduced time to degree, without adversely affecting the number or impact of thesis-related publications (24).

Subsequently, Benzon et al. conducted a retrospective study of the biomedical PhD program *Biology of Neoplasms* at the University of Split School of Medicine, exploring factors associated with the PhD students' completion rates (25). Their findings showed that mentor experience and student employment in academia were significant predictors of successful graduation and higher research output, whereas age, sex, and tuition

support had no measurable impact. Those findings support EU policy recommendations for doctoral program evaluation and reform (25). In 2024, we reported persistent challenges in obtaining the comprehensive list and full texts of PhD theses defended at medical schools in Croatia, highlighting systemic issues in data accessibility and transparency within higher education institutions (5).

Limitations of the Study

Several data inconsistencies and reporting challenges were encountered during the analysis, which may have affected the integrity and interpretability of the dataset. Changes in institutional names and organizational structures complicated longitudinal tracking, making it difficult to ensure consistent institutional identification over time. Inconsistent naming conventions, such as the use of university names versus geographical identifiers, further impeded categorization and limited the potential for automated or programmatic data processing.

Variability in data granularity also posed challenges, as some datasets were reported at the university-wide level while others provided department-level information, complicating fair comparisons across institutions and time periods.

Unclear inclusion criteria for doctorates in the arts further limited disciplinary comparability and undermined international benchmarking. Nonspecific or incomplete entries reduced data granularity and hindered sub-institutional analyses. Additionally, manual data extraction increased the potential for human error, and the absence of standardized formatting, such as alphabetical ordering in earlier datasets, complicated data validation and reduced usability.

A discrepancy identified for the year 2018 underscores the necessity of cross-verifying data from multiple sources and highlights the risks associated with relying on provisional statistics. It also demonstrates the importance of using finalized, validated datasets for accurate analysis. Consequently, researchers should exercise caution when interpreting apparent fluctuations in the number of defended theses, as such variations may result from reporting inconsistencies rather than genuine changes in academic productivity.

Methodologically, this study did not include formal diagnostics for OLS regression assumptions (linearity, homoscedasticity, independence, normality), nor did we employ count-specific models (Poisson, negative binomial, quasi-Poisson) or use standard-error adjustments tailored to counts. As a result, untested data features, such as serial correlation, nonlinearity, or overdispersion, may have affected the precision of estimated standard errors and confidence intervals, potentially leading to over- or under-statement of p-values. Future research could address these limitations by employing count-based or rate-based modeling approaches with robust or quasi-likelihood methods that explicitly account for temporal dependence.

The number of active doctoral programs varies across institutions and over time. This likely influences the total number of theses defended. However, we were unable to adjust for these variations, as consistent, institution-level time series of active programs were not available in the national statistics we analyzed and, to our knowledge, are not publicly traceable for the full period studied. Consequently, the observed trends and between-institution differences reflect both research capacity and institutional availability, rather than productivity per program. Future analyses should incorporate program-level denominators (e.g., number of active programs, enrolled doctoral students, or faculty size) once such data become available.

Future Research

Future research and policy efforts should further investigate the effects of higher education policies and funding mechanisms on doctoral education in Croatia. In particular, analyses should explore how national reforms and the establishment of new doctoral programs have influenced institutional productivity and research quality.

The study period overlaps with two major structural developments. Namely, Croatia accessed the European Union in July 2013. Also, during this period, Croatia adopted the Bologna

Process, which standardized higher education systems across Europe into three academic cycles, including doctoral studies. These milestones likely shaped national research policy, funding priorities and the expansion of doctoral education.

Further studies should also assess the labor market outcomes of PhD graduates in Croatia to evaluate the alignment between doctoral training and national economic and societal needs. Establishing a better data infrastructure within the Croatian Bureau of Statistics would enhance the ability to monitor and evaluate doctoral education. Such improvements would enable evidence-based policymaking, facilitate cross-institutional comparisons, and provide deeper insights into disciplinary trends and program performance.

Additionally, more granular research on PhD education in Croatia is needed, focusing on curriculum development, quality assurance mechanisms, and internationalization strategies. Developing a comprehensive longitudinal dataset on active doctoral programs and student enrollment would allow for rate-based analyses (e.g., theses per program) and provide a more accurate assessment of institutional productivity and efficiency.

Conclusion

This study presents the first comprehensive national analysis of doctoral education in Croatia, documenting substantial growth in doctoral output from 1992 to 2023 and revealing key institutional and disciplinary patterns. The findings underscore the dominant role of the University of Zagreb, the increasing contributions of regional universities, and the sustained prominence of medical, economic, and engineering fields in shaping Croatia's doctoral landscape. These patterns reflect both the historical evolution and ongoing transformation of Croatia's higher education and research systems. As Croatia continues to integrate with European research and education frameworks, maintaining long-term investment in doctoral programs, promoting disciplinary diversity, and supporting the strategic development of emerging institutions will be crucial for strengthening national research capacity and fostering balanced academic advancement.

What Is Already Known on This Topic:
Comprehensive analyses of national trends in the number of defended PhD theses across all academic institutions in Croatia are lacking. Fragmented reports have highlighted issues such as low completion rates and variations in institutional research output, but temporal patterns and institutional comparisons have not been systematically explored. Additionally, the quality and completeness of publicly available data on PhD theses in Croatia remain uncertain, limiting efforts to monitor and evaluate the effectiveness of doctoral education policies over time.

What This Study Adds:
This study provides the first national-level longitudinal analysis of PhD thesis defense trends in Croatia from 1992 to 2023. It identifies leading institutions by output, reveals temporal patterns in doctoral productivity, and highlights significant gaps in data reporting. Notably, the analysis shows that the medical schools, particularly the University of Zagreb School of Medicine, consistently rank among the top institutions in terms of the number of defended PhD theses. These findings offer a foundation for informed policy decisions and future research on doctoral education in Croatia.

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Data Availability: Raw data collected for this study are available on the Open Science Framework (link: https://osf.io/y5wu8/).

Conflict of Interest: The authors declare that they have no conflict of interest.

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Supplementary Material

Table 1. Number of PhD Theses Defended in Croatia by Year (1992-2023)

Year	Number of PhD theses defended
1992	234
1993	267
1994	217
1995	212
1996	352
1997	307
1998	339
1999	338
2000	280
2001	255
2002	314
2003	321
2004	357
2005	368
2006	439
2007	466
2008	494
2009	572
2010	838
2011	1072
2012	1338
2013	830
2014	851
2015	878
2016	646
2017	716
2018	647
2019	680
2020	615
2021	737
2022	792
2023	806

Table 2. Number of PhD Theses Defended in Schools of Medicine in Croatia by Year (1992-2023)

Year	Osijek	Rijeka	Split	Zagreb
1992	-	11	-	52
1993	-	12	-	34
1994	-	9	-	22
1995	-	8	-	34
1996	-	23	-	52
1997	-	21	-	39
1998	-	14	-	64
1999	-	22	-	40
2000	-	10	-	43
2001	-	13	-	41
2002	-	16	-	53
2003	1	12	3	41
2004	4	13	1	41
2005	12	-	7	33
2006	10	18	7	38
2007	16	14	10	37
2008	10	10	9	53
2009	13	24	18	42
2010	20	26	9	82
2011	24	49	16	121
2012	65	59	27	61
2013	47	14	33	69
2014	11	21	26	68
2015	10	10	8	80
2016	11	13	39	90
2017	34	18	13	68
2018	15	20	21	73
2019	22	14	24	79
2020	12	24	17	50
2021	27	10	35	89
2022	22	17	32	59
2023	36	17	51	64

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Breaking the Cycle: A Case-Control Study on Social and Familial Influences in Childhood Obesity

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Abstract

Objective. Childhood obesity is a growing public health concern influenced by social and familial determinants. This study examines the associations between caregiver education, family structure, social risk factors, and familial obesity with childhood obesity in a Portuguese pediatric population to inform targeted interventions. **Materials and Methods.** A retrospective case-control study was conducted at a Portuguese secondary hospital, including 78 children with obesity and 326 controls. Controls were selected using a time-matched, hospital-based approach from the same ward and calendar years as the cases. Socioeconomic data were extracted from the hospital records. Social risk was defined based on documented indicators of socioeconomic vulnerability, such as financial hardship, suspicion of neglect, and housing instability, identified through multidisciplinary records. Logistic regression models were used to assess the risk of obesity while adjusting for age and sex. **Results.** Caregiver education and familial obesity were the strongest predictors of childhood obesity. Children whose caregivers had not completed compulsory education had a significantly higher risk of obesity, whereas familial obesity showed an even stronger association. Social risk factors were linked to obesity in univariate analyses but lost significance in adjusted models. An exploratory interaction between caregiver education and social risk suggested higher odds when both disadvantages co-occurred. Family structure did not independently predict obesity. **Conclusion.** This study highlights the need for targeted public health interventions addressing caregiver education, economic support for at-risk families, and family-wide lifestyle changes. A multi-sectoral approach integrating healthcare, education, and community programs is crucial for reducing childhood obesity and promoting long-term health equity.

Key Words: Caregiver Education ▪ Socioeconomic Factors ▪ Obesity Risk Factors ▪ Health Disparities ▪ Public Health Interventions.

Introduction

Childhood obesity is a major global health concern, with early onset increasing the risk of chronic diseases, such as type 2 diabetes, cardiovascular conditions, and psychological disorders, which often persist into adulthood and burden healthcare systems (1, 2). While lifestyle habits such as diet and physical activity are important, structural factors—such as socioeconomic status, caregiver education, and familial context—play a pivotal role (3). Understanding how these elements interact is key to designing effective and equity-focused interventions.

Children from disadvantaged households often face financial and environmental barriers that increase their risk of obesity. Limited income constrains access to fresh, nutritious foods, whereas affordable options are often processed and calorie-dense (3). These families may also live in areas lacking safe spaces for physical activity or adequate

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food retail infrastructure, reinforcing sedentary behavior and poor diets (3).

Lower parental education is associated with reduced health literacy, limited nutritional knowledge, and inconsistent access to preventive care. These limitations influence early feeding practices and long-term dietary habits. Interventions that promote caregiver education may help families adopt healthier routines and mitigate the risk of obesity. Household composition and social adversity can also influence obesity outcomes (4). Single-parent families often face time constraints and economic pressures, whereas extended or institutional settings may lead to inconsistent caregiving and feeding practices (4). Social risk factors, such as food insecurity and housing instability, contribute to chronic stress, which is linked to emotional overeating and a preference for energy-dense comfort foods. Despite growing awareness, few studies have concurrently examined these determinants in pediatric populations. This case-control study investigates how caregiver education, social risk, and familial obesity relate to childhood obesity within a hospital-based cohort.

By modeling these factors concurrently, this study aims to clarify their independent associations and, where theoretically justified, explore potential joint effects—such as the intersection between social disadvantage and caregiver education—thereby supporting more targeted public health strategies.

Methods

Study Design and Setting

This study employed a retrospective, hospital-based, case–control design conducted at a Portuguese secondary hospital between January 2010 and July 2024. Pediatric inpatients aged 2 to 17 years were considered eligible. All consecutive cases with a documented diagnosis of obesity and complete sociodemographic and clinical data during this period were included, yielding a total of 78 cases. This number reflects the entire population of eligible cases within the defined timeframe, rather than a sampled subset. Controls were selected at an approximate 1:4 ratio from pediatric inpatients aged 2–17 years admitted to the same hospital and ward (Pediatrics) during the same calendar years (2010–2024) as the cases to ensure temporal comparability. No formal matching by age or sex was performed, as these variables were adjusted for in the regression models. Controls were chosen independently of diagnosis, excluding only children with documented obesity to reflect the general inpatient population. Because caregiver education and social risk may be related to a broad range of admission diagnoses, complete independence between exposures and control diagnoses could not be guaranteed; therefore, we used a diagnosis-agnostic approach to preserve representativeness and avoid selection bias from restricting to specific conditions.

Selection Criteria

The selection of cases and controls followed strict inclusion and exclusion criteria to minimize bias. Cases: Children aged 2-17 years with a documented diagnosis of obesity, defined using the WHO BMI-for-age percentiles (5, 6). Admitted to the Pediatrics Department for any medical reason during the study period. Controls: Children aged 2-17 years without an obesity diagnosis. Hospitalized for any non-obesity condition. Selected using a time-matched hospital-based approach to reflect the temporal distribution of cases across the 2010–2024 study period. Controls encompassed a broad range of medical admissions typical of a general pediatric ward, ensuring the representativeness of the inpatient population.

The exclusion criteria for both cases and controls included children with underlying genetic syndromes affecting growth and metabolism, such as Prader-Willi syndrome.

Variables and Definitions

This study analyzed key variables related to socioeconomic and familial factors influencing childhood obesity. Sex was categorized as female or male. Caregiver education was classified into four

levels: higher education (reference), compulsory education, below compulsory education, and illiterate. Compulsory education was defined according to the Portuguese legal framework in force during each caregiver's schooling years (7). Before Law No. 85/2009, compulsory education comprised nine years (basic education); from 2009 onward, it was extended to twelve years (ages 6–18) (7). Accordingly, caregivers' education levels were coded relative to the applicable legal standard for their birth cohort. Family structure was grouped into nuclear (reference), extended, single-parent, and reconstituted household types. Social risk was a binary variable based on documentation of (a) active follow-up by the hospital's child protection/social support team or (b) explicit notes of socioeconomic vulnerability (e.g., economic hardship, suspected neglect, and housing insecurity). No standardized instrument was used. Familial obesity was examined by identifying whether a child had no obese relatives, one direct relative with obesity, or two or more direct relatives with obesity. 'Direct relatives' denotes first-degree family members—parents or primary caregivers (including adoptive parents) and siblings; grandparents and other extended relatives were not considered. Mental health conditions were not analyzed as independent determinants because they were inconsistently documented in the medical records and primarily reflected comorbidities rather than exposures influencing obesity or food access.

Comorbidities

Comorbidities were defined as chronic or recurrent medical conditions documented in the patient's medical history or discharge summary, independent of the reason for hospitalization. Only diagnoses recorded in addition to the admitting diagnosis were included as comorbidities.

Data Collection

Data were extracted from the hospital's electronic medical records. Sociodemographic data, including caregiver education, household composition, and social risk status, were obtained from structured hospital admission interviews conducted by social workers and pediatricians. Anthropometric measurements were recorded following standardized WHO protocols, with BMI percentiles calculated based on sex- and age-specific growth charts (5, 6).

Data on family income, parental employment status, and parental BMI were not systematically recorded in the hospital's electronic medical records and were, therefore, unavailable for analysis. Although these are important factors in understanding childhood obesity, caregiver education and documented social risk were used as proxy indicators for socioeconomic context, and familial obesity was assessed based on the presence of first-degree relatives—parents/primary caregivers (including adoptive) and siblings—with obesity as recorded in medical or social histories. Parental age and ethnicity/ancestry were also not consistently available in the electronic records and were therefore not included as covariates. The hospital's catchment area is >95% Caucasian, indicating limited ethnic heterogeneity across cases and controls.

Ethical Considerations

This study was approved by the Institutional Ethics Committee of the Local Health Unit Entre Douro e Vouga. Given the retrospective nature of this study, the requirement for informed consent was waived. However, all patient data were anonymized to ensure confidentiality, in accordance with the Declaration of Helsinki.

Statistical Analysis

We summarized the baseline characteristics as mean ± SD (continuous) and N (%) (categorical). Univariable logistic regression was used to estimate crude odds ratios (CORs) with 95% confidence intervals (CI). Next, for each exposure, we fitted a minimally adjusted model that included the exposure, age, and sex to obtain age- and sex-adjusted odds ratios (AORs). Finally, we fitted one fully adjusted model, including caregiver education, family structure, social risk, familial obesity, age,

and sex, to assess model discrimination, calibration, and multicollinearity, rather than to estimate effects. Comorbidities were not modeled because several conditions (e.g., asthma and dyslipidemia) plausibly lie on the causal pathway (risk of over-adjustment). Statistical significance was set at two-sided $p < 0.05$. Missing data were <5% per variable, and listwise deletion was used. Multicollinearity was assessed using variance inflation factors (VIF <2 for all predictors). Model discrimination and calibration were evaluated for the full model only using the area under the receiver operating characteristic curve (AUC) and the Hosmer–Lemeshow test. An a priori interaction term (lower caregiver education × social risk) was included in the full model and tested using Wald χ^2. The reference categories were as follows: Sex = Female; Family structure = Nuclear; Caregiver education = Higher education; Social risk = No; Familial obesity = None; Age modeled per 1-year increase. As a sensitivity analysis, we repeated the full model stratified by age [2–5, 6–11, 12–17 years]. Analyses were performed using SPSS v27 (IBM, Armonk, NY, USA).

Results

A total of 78 cases of childhood obesity and 326 controls were analyzed (Table 1). Children with obesity were significantly older than the controls (10.53±0.99 years vs. 7.57±0.52 years, P<0.001). The male-to-female distribution differed between the groups (P<0.01), with a higher proportion of females in the obesity group (60.26%) than in the control group (40.80%).

Table 1. Demographic and Socioeconomic Data of Study Participants

Participants' characteristics	Total (N=404)	Cases (N=78)	Controls (N=326)	P value
Age (years), mean ± SD	8.14±0.48	10.53±0.99	7.57±0.52	<0.001
Sex				
Male	224 (55.45%)	31 (39.74%)	193 (59.20%)	<0.01
Female	180 (44.55%)	47 (60.26%)	133 (40.80%)	
Comorbidities				
No	156 (38.61%)	21 (26.92%)	135 (41.41%)	0.02
Yes	248 (61.39%)	57 (73.08%)	191 (58.59%)	
Family structure				
Nuclear	301 (74.50%)	52 (66.67%)	249 (76.38%)	0.22
Extended	47 (11.63%)	9 (11.54%)	38 (11.66%)	
Single-parent	36 (8.91%)	11 (14.10%)	25 (7.67%)	
Reconstituted	20 (4.95%)	6 (7.69%)	14 (4.29%)	
Caregiver education level				
Higher education	100 (24.75%)	6 (7.69%)	94 (28.83%)	<0.001
Compulsory education	200 (49.50%)	39 (50.00%)	161 (49.39%)	
Below compulsory education	104 (25.74%)	33 (42.31%)	71 (21.78%)	
Illiterate	-	-	-	
Social risk				
No	373 (92.33%)	62 (79.49%)	311 (95.40%)	<0.001
Yes	31 (7.67%)	16 (20.51%)	15 (4.60%)	
Familial obesity				
No	340 (84.16%)	39 (50.00%)	301 (92.33%)	<0.001
One direct family member	46 (11.39%)	28 (35.90%)	18 (5.52%)	
Two or more direct family members	18 (4.46%)	11 (14.10%)	7 (2.15%)	

Continuous variables are expressed as mean ± SD; categorical variables are N (%) using column percentages. P values are two-sided (t test for age; χ^2 or Fisher's exact for categorical variables, as appropriate). The "Illiterate" category had zero counts in both groups and was excluded from χ^2 testing (shown for completeness). SD=Standard deviation.

Comorbidities in Obese and Non-Obese Children

Children with obesity exhibited a significantly higher prevalence of comorbidities than controls (73.08% vs. 58.28%, P=0.02). The most frequently reported conditions were respiratory diseases, including asthma and recurrent wheezing, which were more prevalent in the cases. Neurodevelopmental disorders, such as attention-deficit/hyperactivity disorder (ADHD) and learning disabilities, were similarly distributed between the groups. Endocrine disorders, including dyslipidemia and metabolic syndrome, were markedly more frequent among children with obesity, reinforcing the well-documented metabolic consequences of excess weight in pediatric populations.

Impact of Caregiver Education on Childhood Obesity

Caregiver education was a significant predictor of obesity in children (Table 2). Compared with children whose caregivers had higher education levels, those whose caregivers had only completed compulsory education had an odds ratio (AOR) of 3.64 (95% CI: 1.31-10.13, P=0.01). Children whose caregivers had below compulsory education had an even higher AOR of 4.76 (95% CI: 1.64-13.85, P<0.01). These findings support previous evidence that lower caregiver education is associated with reduced nutritional knowledge, lower health literacy, and limited access to healthcare resources. Table 2 reports crude odds ratios from univariable analyses and adjusted odds ratios from models controlling for age and sex.

Table 2. Logistic Regression Analyses for Childhood Obesity (Crude and Age/Sex-Adjusted)

Variable	Categories	Univariate		Adjusted	
		COR (95% CI)	P value	AOR (95% CI)	P value
Age	-	1.13 (1.07-1.19)	<0.001	1.08 (1.01-1.15)	0.02
Sex	Female	Reference	-	Reference	-
	Male	0.46 (0.27-0.75)	<0.01	0.58 (0.32-1.05)	0.07
Family structure	Nuclear	Reference	-	Reference	-
	Extended	1.13 (0.52-2.49)	0.75	0.78 (0.29-2.09)	0.62
	Single-parent	2.11 (0.98-4.55)	0.06	1.28 (0.47-3.49)	0.63
	Reconstituted	2.21 (0.80-6.08)	0.13	1.21 (0.36-4.15)	0.76
Caregiver education level	Higher education	Reference	-	Reference	-
	Compulsory education	3.80 (1.55-9.30)	<0.05	3.65 (1.31-10.13)	0.01
	Below compulsory education	7.28 (2.89-18.32)	<0.001	4.76 (1.64-13.85)	<0.01
Social risk	No	Reference	-	Reference	-
	Yes	5.35 (2.51-11.39)	<0.001	2.63 (0.97-7.09)	0.06
Familial obesity	No	Reference	-	Reference	-
	One direct family member	12.01 (6.09-23.69)	<0.001	8.04 (2.73-23.65)	<0.001
	Two or more direct family members	12.13 (4.44-33.12)	<0.001	9.74 (4.63-20.49)	<0.001
Interaction term	Lower caregiver education × social risk	-	-	4.03 (1.50-10.80)	<0.05

COR=Crude odds ratio from univariable models; AOR=Age- and sex-adjusted odds ratio from separate models that include the listed predictor + age (continuous, per 1-year) + sex; Interaction term (caregiver education ≤ compulsory vs higher × social risk yes/no) was estimated in a model including age, sex, caregiver education, social risk, and their product; P values are two-sided; report exact values (use <0.001 when smaller); CI=confidence interval.

Influence of Social Risk on Obesity Prevalence

Social risk, defined by factors such as economic hardship and food insecurity, was associated with obesity, although the adjusted model showed marginal significance (AOR=2.63, 95% CI: 0.97-7.09, P=0.06). Despite not reaching statistical significance in the adjusted analysis, the univariate analysis showed a strong association (COR=5.35, 95% CI: 2.51-11.39, P<0.001), reinforcing the role of socioeconomic disparities in the prevalence of obesity (Table 2). These findings align with prior research showing that financial constraints may limit access to fresh foods and promote reliance on calorie-dense processed foods (8, 9).

Effect of Family Structure on Obesity

Family structure did not show a statistically significant effect on childhood obesity after adjusting for confounders (P>0.05) (4). Although previous studies suggest that children from single-parent households may be at higher risk due to time constraints affecting meal preparation and supervision of physical activity, no significant association was found in the present analysis (Table 2).

Role of Familial Obesity

Familial obesity was the strongest predictor of obesity in children (Table 2) (10). Children with one direct relative with obesity had an AOR of 8.04 (95% CI: 2.73-23.65, P<0.001), while those with two or more direct relatives with obesity had an even higher AOR of 9.74 (95% CI: 4.63-20.49, P<0.001) (10). These results reinforce the impact of genetic predisposition and shared environmental factors on the risk of obesity (10).

Multivariable Analysis and Interaction Effects

After adjusting for multiple variables, caregiver education and familial obesity remained significant predictors of childhood obesity, whereas family structure and social risk were not statistically significant in the adjusted model. Interaction analysis indicated that children from low-education households with high social risk had a high likelihood of obesity (AOR: 4.03, 95% CI: 1.50-10.80, P<0.05), suggesting a compounded effect of socioeconomic disadvantage. The final multivariable model demonstrated good discrimination (AUC=0.832, 95% CI: 0.780–0.884, P<0.001) and adequate calibration (Hosmer–Lemeshow χ^2=7.95, df=8, P=0.44), indicating reliable model fit. Age-stratified sensitivity analyses (2–5, 6–11, and 12–17 years) confirmed the stability of the associations across developmental stages. Familial obesity remained the strongest determinant across all age groups, whereas lower caregiver education showed increasing effects with age. Social risk and family structure retained similar directions but did not achieve statistical significance. The full stratum-specific results are presented in Supplementary Table S1.

Discussion

This study reinforces the profound impact of social determinants on childhood obesity, aligning with the existing literature while highlighting critical intervention points. Caregiver education and familial obesity emerged as the most influential factors, with children from families with lower educational attainment or a history of obesity facing significantly higher risks. While social risk factors were strongly associated with obesity in the univariate model, their influence was reduced in the adjusted analysis, suggesting that they may operate through intermediary pathways. The absence of a significant association between family structure and obesity aligns with some prior studies but contrasts with research indicating an increased risk among children from single-parent households (4). These findings underscore the need for targeted, multi-level public health interventions.

Supplementary Table. Age-Stratified Multivariable Logistic Regression Models for Determinants of Childhood Obesity

Variable	Categories	AOR (95% CI)	P-value	AOR (95% CI)	P-value	AOR (95% CI)	P-value
		Ages 2–5 years (N=158)		Ages 6–11 years (N=135)		Ages 12–17 years (N=111)	
Caregiver education	Higher	Reference	-	Reference	-	Reference	-
	Compulsory	2.42 (0.44–13.29)	0.31	7.85 (1.10–55.87)	0.04	8.38 (0.70–100.55)	0.09
	Below compulsory	3.05 (0.47–19.70)	0.24	5.97 (0.74–47.97)	0.09	20.25 (1.48–277.61)	0.02
Social risk	No	Reference	-	Reference	-	Reference	-
	Yes	4.66 (0.60–36.15)	0.14	1.69 (0.25–11.26)	0.59	1.04 (0.20–5.42)	0.96
Family structure	Nuclear	Reference	-	Reference	-	Reference	-
	Single-parent	NE*	NE*	0.33 (0.02–4.61)	0.41	4.29 (1.04–17.75)	<0.05
	Extended	0.20 (0.01–3.33)	0.26	0.61 (0.09–4.15)	0.61	1.91 (0.32–11.30)	0.48
	Reconstituted	NE*	NE*	1.54 (0.23–10.15)	0.66	1.73 (0.23–13.29)	0.60
Familial obesity	No	Reference	-	Reference	-	Reference	-
	One affected relative	4.90 (0.94–25.37)	0.06	61.17 (9.36–399.83)	<0.001	9.76 (2.65–36.01)	<0.001
	≥Two affected relatives	20.39 (1.48–280.85)	0.02	10.59 (1.69–66.19)	0.01	5.52 (1.01–30.09)	<0.05
Sex	Female	Reference	-	Reference	-	Reference	-
	Male	0.34 (0.09–1.26)	0.11	0.72 (0.24–2.15)	0.56	0.64 (0.23–1.79)	0.40
Model fit†	-	AUC=0.83; Hosmer–Lemeshow P=0.95	-	AUC=0.86; Hosmer–Lemeshow P=0.57	-	AUC=0.75; Hosmer–Lemeshow P=0.79	-

Within each age stratum, models adjust for caregiver education, family structure, social risk, familial obesity, and sex (age omitted within stratum). *Not estimable because of sparse cells or quasi-complete separation (estimate unstable/non-convergent); category retained for transparency (see Table 1 for counts). †AUC and Hosmer–Lemeshow P are reported for model performance; AOR=Adjusted odds ratio; CI=Confidence interval.

Education and Economic Hardship: The Double Burden on Childhood Obesity

Lower caregiver education levels and economic hardship significantly increased the risk of childhood obesity, consistent with previous studies linking these factors to reduced nutritional knowledge, limited healthcare access, and higher reliance on processed foods (8, 9, 11). Children with caregivers who had not completed compulsory education exhibited nearly five times the risk of obesity compared to those from higher-education households (AOR=4.76, 95% CI: 1.64-13.85, P<0.01). Similarly, food insecurity and financial constraints shape access to nutritious food and opportunities for physical activity, thereby exacerbating the risk of obesity (8). Although social risk factors were strongly associated with obesity in the univariate analyses, their influence diminished after adjusting for confounders (AOR=2.63, 95% CI: 0.97-7.09, P=0.06). This suggests that, although social risk may not act as a direct predictor, it is a key contextual factor in shaping childhood obesity outcomes (3). The education × social-risk interaction suggests compounded vulnerability when structural and educational disadvantages co-occur, mirroring the mechanisms seen in complex social determinant frameworks.

Given the robust association observed in the unadjusted models, further research should explore how financial constraints contribute to unhealthy dietary habits and stress-related eating behaviors (12). Policymakers should integrate economic support measures, such as food subsidies, school meal programs, and community-based nutrition initiatives, into obesity prevention strategies to reduce socioeconomic disparities in childhood obesity.

Rethinking Family Structure: A Lesser Role in Obesity Risk?

In contrast to some prior studies, family structure did not independently predict childhood obesity in the adjusted models (4). Children from single-parent households had higher obesity odds in the univariate analysis; however, this association lost statistical significance in the final model (AOR=1.28, 95% CI: 0.47-3.49, P=0.63). This suggests that broader socioeconomic variables, such as income stability and caregiver education, may have a stronger influence on the risk of obesity than household composition alone. Future research should explore how parenting dynamics, meal patterns, and home environments interact with obesity risk, rather than focusing solely on family structure as a risk factor (13).

The Family Factor: How Genetics and Environment Converge on Obesity

Familial obesity remained the strongest predictor of childhood obesity in both the univariate and adjusted analyses. Children with one obese relative had an eight-fold increased risk of obesity, while those with two or more obese relatives had nearly a ten-fold increase (AOR=9.74, 95% CI: 4.63-20.49, P<0.001) (10). These findings reaffirm the complex interplay between genetic susceptibility and shared environmental influences. Given the significance of familial obesity, prevention strategies should prioritize family-wide interventions that encourage healthier behaviors across generations (14). Evidence suggests that structured, multi-generational lifestyle programs are among the most effective in reducing obesity risk (15).

From Research to Action: Public Health Strategies for Obesity Prevention

The findings of this study emphasize the necessity of multifaceted interventions that address both the individual and structural determinants of childhood obesity. Policymakers should prioritize initiatives aimed at reducing socioeconomic disparities, enhancing access to affordable and nutritious foods, and integrating comprehensive health education into early childhood development programs. Given the strong influence of familial obesity, interventions should adopt a family-centered approach rather than focusing solely on the child (14). Additionally, community-driven initiatives that promote physical activity and provide nutritional support can help mitigate the rising obesity epidemic among at-risk populations (3).

Beyond population-level strategies, these findings also highlight opportunities for family-centered interventions within pediatric and primary care settings. Given the strong familial clustering of obesity, programs that actively engage caregivers—biological or adoptive—and siblings in shared behavior change are likely to yield greater and more sustainable results. Integrating parental health literacy counseling, practical nutrition and activity guidance, and psychosocial support for families facing socioeconomic adversity can translate research evidence into everyday practice. Partnerships between healthcare teams, schools, and community organizations should prioritize coordinated education and empowerment of entire households, addressing both knowledge gaps and the social constraints that shape children's health behaviors.

While the association between socioeconomic status and childhood obesity has been widely studied, this study adds to the literature by using a case-control design in a Southern European hospital setting and integrating caregiver education, social risk, family structure, and familial obesity into a single multivariable model. The identification of a compounded effect between low caregiver education and social risk offers novel insights into the structural dynamics of obesity risk in this context.

Limitations and Future Research: Filling the Gaps

While this study offers valuable insights into the social determinants of childhood obesity, several limitations must be acknowledged. First, reliance

on hospital inpatient data may introduce selection bias, as the study population may not fully reflect the general pediatric population. Additionally, because controls were selected agnostically with respect to admission diagnosis, we cannot exclude associations between exposures (caregiver education, social risk) and control diagnoses; this could bias effect estimates, although our inclusive strategy reduces bias from restricting to specific conditions. Second, behavioral factors (screen time, physical activity, and dietary habits) were not comprehensively captured; conceptually, these lie on the causal pathway from socioeconomic exposures to obesity and thus function as mediators rather than confounders. Therefore, their omission is unlikely to constitute residual confounding in our models. Data on children's and caregivers' mental health were also incomplete and therefore excluded from multivariable models. Although psychological factors may influence eating behaviors and access to food, they were beyond the scope of this retrospective analysis. In contrast, family income, parental employment status, parental BMI, and parental age—plausible confounders likely associated with both exposures and obesity—were unavailable and may have introduced residual confounding. Ethnicity/ancestry data were also unavailable; given the catchment's >95% Caucasian profile, material confounding by ethnicity is unlikely. Although the analysis adjusted for age as a continuous variable, the broad age range of 2 to 17 years encompasses distinct developmental stages. Future research may benefit from stratifying by age group to better capture stage-specific obesity risk patterns. Longitudinal studies tracking obesity trajectories from childhood to adulthood would further illuminate the long-term effects of socioeconomic determinants on weight status (16). Additionally, qualitative research exploring parental perspectives on obesity-related barriers and motivators could enhance the effectiveness of public health interventions aimed at preventing obesity in early life (13).

Conclusion

This study underscores the powerful influence of caregiver education and familial obesity on childhood obesity, while also recognizing the intricate role of social risk factors. Addressing these determinants through targeted interventions, such as parental health literacy programs, economic assistance for at-risk families, and structured family-wide lifestyle modifications may significantly contribute to obesity prevention. Given the multifactorial nature of obesity, a broad public health approach that integrates school-based initiatives, regulatory policy changes, and community outreach programs is crucial for achieving sustainable reductions in childhood obesity rates (14). By adopting multi-sectoral strategies, policymakers can ensure equitable health opportunities and foster long-term well-being for children vulnerable to obesity.

What Is Already Known on This Topic:

Childhood obesity is a multifactorial condition that is strongly associated with socioeconomic disparities and familial factors. Lower caregiver education levels, social vulnerabilities, and household dynamics influence children's dietary habits and access to healthy environments. Familial obesity is a recognized risk factor that reflects the combined influence of genetic predisposition and shared lifestyle behaviors.

What This Study Adds:

This case–control study identified caregiver education and familial obesity as the strongest predictors of childhood obesity, whereas social risk showed a weaker association that attenuated after adjustment and did not reach statistical significance. Considering these factors jointly, the co-occurrence of low caregiver education and social risk was associated with higher odds of obesity than either factor alone. These findings underscore the importance of targeted, family-centered interventions and socioeconomic support strategies to reduce childhood obesity.

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Literature Review
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Ansa Pancreatica: Clinical Significance in Recurrent Acute Pancreatitis

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Abstract

Objective. This study aimed to conduct a thorough literature review regarding the ansa pancreatica as a potential risk factor for recurrent acute pancreatitis, exploring its pathophysiological mechanisms and possible complications during the surgical management of pancreatic conditions. **Methods.** A comprehensive search was performed in the PubMed and Scopus databases using the keyword 'Ansa Pancreatica,' yielding a total of 80 articles (PubMed: 34, Scopus: 46, with 52 unique articles). After applying strict inclusion and exclusion criteria, unrelated and duplicate articles were removed, resulting in the selection of 38 relevant studies. **Results.** Ansa pancreatica was found to be a statistically significant independent risk factor for recurrent acute pancreatitis in the majority of the literature reviewed. The suggested pathophysiological mechanism involves anatomical obstruction and subsequent pre-activation of the pancreatic enzymes, causing an inflammatory cascade. Diagnosis can be established using Endoscopic Retrograde Cholangiopancreatography, Magnetic Resonance Cholangiopancreatography, or Endoscopic Ultrasonography, while treatment options are either conservative or surgical, with the invasive procedures being associated with a significant risk of complications. Furthermore, some studies have indicated a correlation between ansa pancreatica and intraductal mucinous neoplasms. **Conclusion.** The findings clearly show that Ansa Pancreatica is a rare anatomical variant with significant clinical and surgical implications, underscoring the necessity for clinicians to be aware of it to mitigate complications and effectively manage pancreatic diseases.

Key Words: Ansa Pancreatica ▪ Pancreatitis ▪ Recurrent Pancreatitis ▪ Pancreatic Ductal Anomalies.

Introduction

The term ansa originates from the Latin word ānsa, meaning handle or loop. This makes it the appropriate term for describing the anatomical variant in question, characterized by an S-shaped looping duct that branches from the duct of Wirsung, linking it to the accessory duct, and ending at or near the minor papilla. Studies from the 20th century have already linked ansa to recurrent acute pancreatitis (1), an inflammatory disease of the pancreas and a frequent emergency faced by general surgery departments (2). Ansa pancreatica constitutes a clinical challenge, as lack of awareness can hinder timely diagnosis and management of the underlying anatomical anomaly, resulting in complications and prolonged hospitalization (3).

The aim of this literature review is to provide a comprehensive and current examination of ansa pancreatica. More specifically, it seeks to explore the relationship between this variant and recurrent acute pancreatitis, review recent findings connecting Ansa with certain neoplasms, and present available imaging modalities alongside up-to-date surgical approaches for managing acute pancreatitis.

Materials and Methods

In June 2025, a comprehensive search was performed in the PubMed and Scopus databases using the keyword 'Ansa Pancreatica.' This search initially yielded 80 articles (34 from PubMed and 46

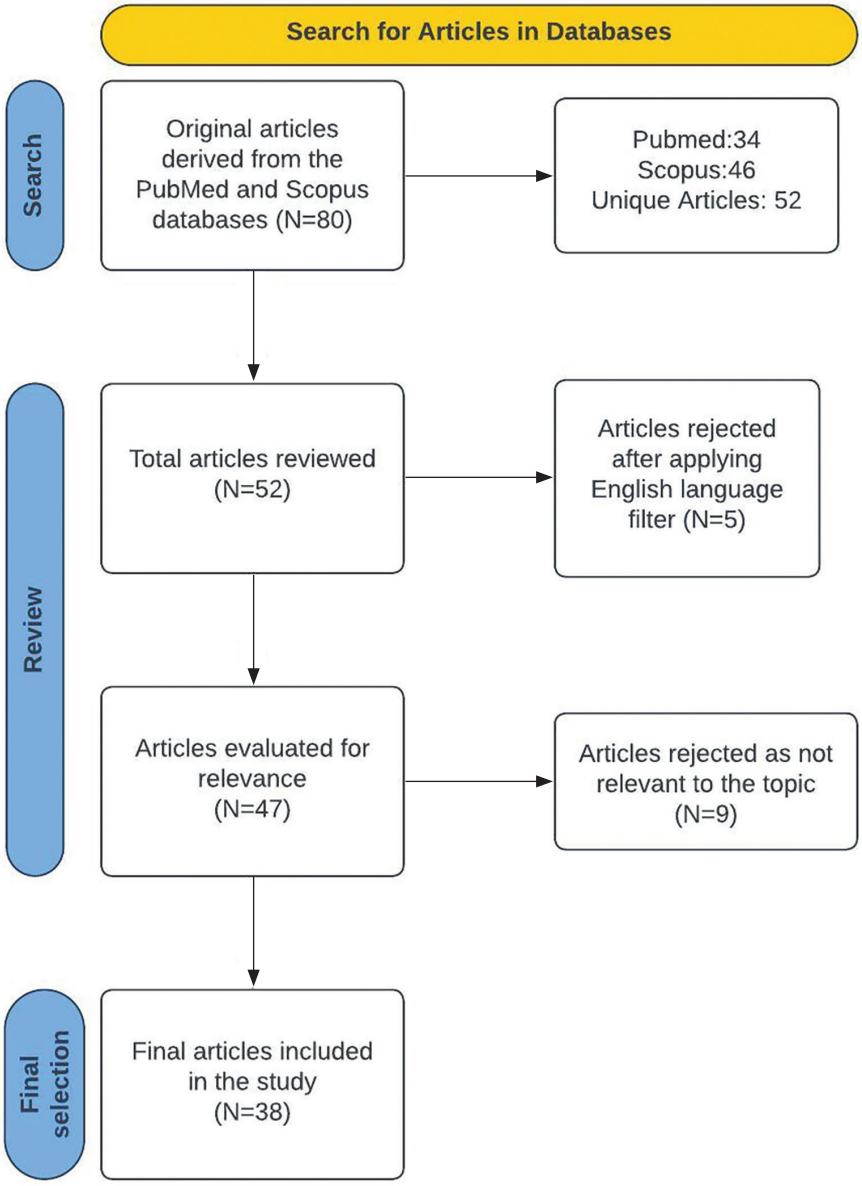

Figure 1. Flow chart of the literature search.

from Scopus, resulting in 52 unique entries). After applying specific inclusion and exclusion criteria (1. English Language and 2. Relevance), unrelated and duplicate articles were removed, leading to the final selection of 38 articles published between 1961 and 2024 (Figure 1).

Results

The review comprises 38 articles that include case studies (22), cohort studies (10), and literature reviews (6) (Table 1). Among the case studies, 3 focus on the Ansa variant, 3 link Ansa pancreatica to tumorigenesis, 13 examine the connection, or potential connection, between Ansa and pancreatitis, 4 discuss surgical complications in patients with the Ansa variant, and 1 questions the prevailing understanding of Ansa. The ages of patients in these case reports ranged from 11 to 80 years, with an average age of 46.4 years.

Table 1. Cited Studies (Reviews and Cohort Studies)

Title of review studies	Year	Author
Clinical importance of main pancreatic duct variants and possible correlation with pancreatic diseases	2020	Dugic A, et al.
Ansa pancreatica as a rare cause of pancreatitis: A review of case reports	2024	Bukowski JS, et al.
Ansa pancreatica. review of the literature	2019	Sotirios K, et al.
Development of the human pancreas and its exocrine function	2022	Mehta V, et al.
Pancreatitis in the developmentally anomalous pancreas	2020	Wood CG, et al.
Endoscopic ultrasound in pancreatic duct anomalies	2023	Chatterjee A, et al.
Title of cohort studies		
An anatomical-radiological study on the pancreatic duct pattern in man	1961	Dawson W, et al.
Accessory pancreatic duct patterns and their clinical implications	2015	Prasanna LC, et al.
Pancreatic ductal morphological pattern and dilatation in postoperative abdominal pain in patients with congenital choledochal cyst: an analysis of postoperative pancreatograms	2000	Koshinaga T, et al.
Anatomic variations of the pancreatic duct and their relevance with the cambridge classification system: MRCP findings of 1158 consecutive patients	2016	Adibelli ZH, et al.
Ansa pancreatica as a predisposing factor for recurrent acute pancreatitis	2016	Hayashi TY, et al.
Branch Fusion Between the Ventral and Dorsal Pancreatic Duct	1994	Hirooka T, et al.
Fusion variations of pancreatic ducts in patients with anomalous arrangement of pancreaticobiliary ductal system	1998	Ishii H, et al.
Groove pancreatitis: Endoscopic treatment via the minor papilla and duct of santorini morphology	2017	Chantarojanasiri T, et al.
Anatomical pancreatic variants in intraductal papillary mucinous neoplasm patients: a cross-sectional study	2022	Johansson K, et al.
Anatomical patterns of the pancreatic ductal system - A cadaveric and magnetic resonance cholangiopancreatography study	2019	Prasad M, et al.

Of the 6 reviews, 3 confirm a significant association between Ansa pancreatica and pancreatitis, while 2 suggest a possible link. The final review emphasizes the benefits of endoscopic ultrasound (EUS) in diagnosing pancreatic duct irregularities, including Ansa. Among the 10 cohort studies, 4 connect the variant with pancreatitis, 5 characterize the variant itself, and 1 identified an elevated risk for multiple cystic lesions in patients presenting with papillary mucinous neoplasm alongside Ansa pancreatica.

Discussion

Embryologically, the pancreas develops from two endodermal buds, the ventral and dorsal buds, which appear during the fifth week of embryonic development. The ventral bud differentiates into the head and uncinate process, while the dorsal bud gives rise to the neck, body, and tail. Consequently, the dorsal bud forms the duct of Santorini, and the duct of Wirsung is formed by both buds—its proximal third from the ventral bud and the distal two-thirds from the dorsal bud (3).

As previously described, Ansa forms through the merging of an inferior branch of the main pancreatic duct (MPD) with either an inferior branch of the proximal accessory pancreatic duct (APD) or directly with the proximal part of the APD (4-6) (Figure 2).

The formation of the S-shaped loop may serve to alleviate drainage issues caused by the obstruction of the APD near its junction with the MPD by connecting the two ducts (7). However, evidence indicates the presence of two subtypes in terms of the patency of the APD: one with a patent duct of Santorini leading to the duodenum (minor

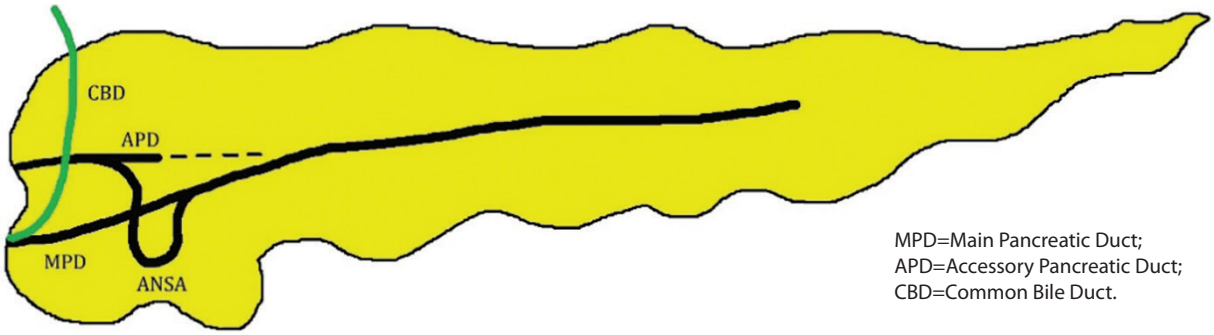

Figure 2. Ansa Pancreatica.

papilla) and one without (6-8). Most studies report that the non-patent type is the more common variant, comprising approximately 55.5% to 80% of cases (6, 7, 9).

Notable discrepancies in the literature warrant attention. Some authors classify the patent subtype as a distinct ductal anomaly (8). Additionally, Guerroum et al. described a case of Ansa with a non-patent major papilla (10), while Koshinaga et al. stated that all Ansa cases with congenital choledochal cysts presented with a radiologically patent APD and dilated ducts (11). These controversies underscore the necessity for additional research concerning whether these sub-variants should be included within or excluded from the Ansa spectrum.

First identified by Dawson and Langman in 1961 (6), Ansa pancreatica is the rarest variation of pancreatic ducts, with its true incidence still uncertain. Among various studies, the reported incidence varies considerably, ranging from 0.25% to 22.5% (12, 7). The maximum figure of 22.5% deviates significantly from the rest of the findings, in which the maximum reported incidence is 6,7%, and derives from an Indian cadaveric cohort study (7), underscoring a potential correlation between ethnicity and the occurrence of this variant. In a significant retrospective study by Abidelli et al. involving 1158 patients, the incidence of Ansa was found to be 1.2% (13), while Hayashi et al. reported rates of 0.85% within a community group (14). Neither study found sex to influence the variation's prevalence. It is crucial to note that both employed MRCP as their imaging modality, which may slightly underestimate Ansa's true occurrence compared to ERCP or surgical investigations, despite being the only non-invasive option for healthy individuals (13, 14).

In our investigation, we encountered an alternative presentation of Ansa, where a looping duct intersects the duct of Wirsung as it connects with the duct of Santorini (12, 14). In this contentious variant, the APD is present but does not contribute to the loop formation, which entirely arises from the MPD or its branches. Additionally, we identified two studies that contest the existence of Ansa pancreatica. In their case report, Suda et al. indicated no evidence of fusion between two inferior branches, suggesting instead that Ansa arises solely from the APD with the MPD merging directly into the APD (15). They did, however, affirm the merger of an inferior branch from the dorsal pancreatic duct (APD) with the ventral duct (MPD). While Dawson and Langman (6) initially defined Ansa as resulting from two inferior branches from the MPD and APD, many researchers consider the direct merging of an inferior branch from the MPD into the APD as characteristic of Ansa, as the S-shaped loop is present in those cases too (5, 8, 9). This finding may be seen as an additional subvariant.

Hirooka et al., in their cohort study, reported a branch originating from the MPD following the expected curvature and terminating at the minor papilla, without any evidence of the APD or its branches, thereby questioning the validity of Ansa (16). Nonetheless, it should be noted that the sample lacked histopathological examination,

which may undermine the findings' reliability. Clearly, further research is essential to elucidate the extensive range of Ansa's subvariants, as a consensus on many subtypes remains elusive, primarily due to their infrequency. Future research could also examine the clinical impact of each of these subvariants, given that the current literature is already limited and predominantly focuses on the most frequent and academically endorsed subtypes.The relevance of Ansa pancreatica is underscored by its relationship with pancreatitis. The majority of the literature indicates that Ansa serves as a predisposing (5, 13, 14, 17, 19) or a potential predisposing factor (2, 7, 9, 20- 22) for pancreatitis. Ishii et al., in their cohort study, found that approximately 7% of patients with this anatomical variation experienced acute pancreatitis (1), while Hirooka et al. reported a uch higher incidence of 80% (4 out of 5) (16). Hayashi et al. established that the occurrence of Ansa was notably elevated (11.1%) among patients with recurrent acute pancreatitis, indicating a 20% risk for those with Ansa, which provides strong statistical evidence (14).

The association between Ansa and pancreatitis has been well-documented, with numerous authors linking it to: recurrent acute pancreatitis (5, 14, 16, 17, 19), acute pancreatitis (14, 19, 20), alcoholic pancreatitis (5, 17, 23), walled-off pancreatitis (17, 23), pancreatitis due to functional stenosis of the sphincter of Oddi (10), and even groove pancreatitis (24). Furthermore, Hussain S.N.F. et al. discussed the case of an 11-year-old patient whose acute pancreatitis was attributed to Ansa pancreatica, thereby including it in the differential diagnosis for the pediatric age group (25). In this demographic, acute pancreatitis is associated with high mortality and morbidity rates, making early diagnosis crucial for favorable outcomes.

The suggested pathophysiological mechanism for pancreatitis onset involves the obstruction of pancreatic secretion flow. Specifically, the looped duct meets the main pancreatic duct (MPD) at an oblique angle, resulting in increased intraductal pressure and early activation of pancreatic enzymes, which subsequently digest pancreatic tissue, trigger an inflammatory response, and lead to pancreatitis (3, 8, 12, 26). Additionally, a possibly non-functional duct of Santorini draining into the minor papilla may exacerbate the already insufficient drainage (8).

Recent studies have highlighted some connections between Ansa pancreatica and intraductal pancreatic mucinous neoplasm (IPMN) or major papilla adenoma (27-29). The first cohort study exploring the relationship between Ansa pancreatica and IPMN found a significant association linking Ansa with the presence of multiple cysts in IPMN patients, a known high-risk factor for concurrent pancreatic ductal adenocarcinoma (30). However, more research is warranted to clarify the causal links.

Lee S.-W. et al. reported a case involving concurrent gallbladder agenesis, Ansa, and Santorinicoele (31). While it is established that obstruction of the ductal wall plays a role in Santorinicoele's pathogenesis, it remains unclear whether Ansa pancreatica or gallbladder agenesis has a causal relationship. In clinical practice, ansa pancreatica can be diagnosed via Endoscopic Retrograde Cholangiopancreatography (ERCP), Magnetic Resonance Cholangiopancreatography (MRCP), and Endoscopic Ultrasonography (EUS) (32). ERCP is regarded as the gold standard imaging technique for this variation, though the sigmoid (S-shaped) branch of Ansa pancreatica may be misidentified as annular pancreas during imaging. Distinctions can be made through the pancreatogram—annular pancreas typically encircles the duodenum, while Ansa's looping branch stays within the pancreatic confines and does not cross the duodenum (8).

MRCP is a non-invasive modality for assessing pancreatic ducts, presenting a safer alternative to ERCP, as it can identify malignancies and carries a lower risk of complications (13). However, despite improvements in imaging, MRCP may miss cases of Ansa when compared to ERCP, as it detects larger ducts with significant pancreatic secretion congestion (14). Nevertheless, Abidelli et al. suggest that the accuracy of ERCP and MRCP is approximately equivalent (13). Notably, Shaikh et al. provided the initial imaging of the variation using EUS, which poses fewer complications than ERCP and has a higher accuracy than

MRCP (8). Consequently, the authors advocate for utilizing EUS when MRCP results are negative before resorting to ERCP. However, there is still no unanimous agreement on the superiority of any diagnostic tool presently (32). The treatment approaches for pancreatitis arising from Ansa pancreatica are still debated. Given the potential for serious iatrogenic complications, the selection of patients for endoscopic management must be meticulous, after careful evaluation of their risk-benefit ratio (3).

Sphincterotomy and/or stent placement in the pancreatic duct remain the most commonly employed strategies (12). Sphincterotomy may target the major papilla, the minor papilla, or both, improving pancreatic flow dynamics, and is promising in reducing pancreatitis recurrence (12, 19, 33). An alternative to sphincterotomy is botulinum toxin injection, albeit used less frequently (34). Surgical pancreatico-jejunostomy has been suggested by Guerroum et al. (10) for non-patent major papilla cases. If endoscopic cannulation for sphincterotomy becomes technically challenging due to Ansa (33, 34), a Rendez-Vous technique is recommended (23, 35, 36). This approach may be conducted as a transgastric procedure with ultrasound assistance (36) or via a transpapillary (retrograde) method (23, 35), depending on anatomical and technical factors. If all other methods fail to avert pancreatitis recurrence, endoscopic ligation of the Ansa deformity might be a consideration (19).

In order to avoid the significant risk of complications associated with interventional techniques, Harbi H. et al. opted for a conservative management strategy, employing pancreatic enzyme replacement therapy (pancrelipase) to decrease pancreatic secretions and, consequently, lower intraductal pressure (37). This approach successfully prevented acute pancreatitis recurrence during a two-year follow-up, thus being added to potential treatment strategies, although its therapeutic effectiveness remains unproven. Given the significant complications resulting from an unrevealed Ansa, such as post-ERCP pancreatitis (38), some authors recommend preoperative screening when there is a substantial suspicion of pancreatic duct abnormalities (1) or multiple occurrences of pancreatitis without an identifiable cause (8). Additionally, Ha J. et al. proposed screening for ductal variations like Ansa in all cases of recurrent pancreatitis localized in the head or uncinate process of the pancreas (4).

Conclusion

This literature review aims to offer a comprehensive and systematic examination of the uncommon anatomical structure known as Ansa pancreatica. The analysis of current data demonstrated ansa pancreatica as an independent risk factor for recurrent acute pancreatitis, primarily due to the obstruction of the flow of pancreatic secretions. Furthermore, emerging studies associating the variant with IPMN warrant further attention. The available imaging techniques for assessing Ansa pancreatica comprise ERCP, MRCP, and EUS, while the treatment strategies are either conservative or interventional, with the invasive approach carrying a significant risk for complications. As a result, healthcare professionals should consider it as a potential differential diagnosis to facilitate accurate diagnosis, select the appropriate management strategy, and minimize the risk of procedural and disease-related complications.

What Is Already Known on This Topic: *Existing literature has already described the various subtypes of Ansa pancreatica, established its clinical relevance, and outlined the available imaging and surgical techniques reported to date. However, given the rarity of Ansa and the fact that a considerable portion of the available data and evidence derives from isolated case reports, consensus regarding its subtypes and optimal management remains elusive.*

What This Study Adds:
The aim of the present study is to investigate the full spectrum of the suggested subvariants of Ansa Pancreatica and their clinical implications, particularly pancreatitis and possible association with IPMN or major papilla adenoma. Additionally, the study summarizes the current imaging modalities and treatment approaches, either surgical or conservative.

Authors' Contributions: Conception and design: ASak; Acquisition, analysis and interpretation of data: ASak; Drafting the article: ASak and DF; Revising it critically for important

intellectual content: ASak, ASh, ASam and DF; Approved final version of the manuscript: ASak, ASh, ASam, NS and DF.

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Mini-Review
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Connections Between Prefrontal Cortex Anatomy and Autism Spectrum Disorder: A Literature Review

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Abstract

Objective. This review examines the existing literature on the structural and functional changes in the anatomy of the prefrontal cortex (PFC) associated with autism spectrum disorder (ASD), focusing on the roles of molecular signaling disruptions and trace element imbalances. **Methods.** A literature review was performed through a structured search of academic publications from 2010 to 2025. **Discussion.** Anatomic variations and structural and functional abnormalities within the PFC, including disruptions in neural connectivity, synaptic plasticity, and neurochemical balance, significantly contribute to the cognitive, social, and emotional deficits observed in ASD. The interplay between brain-derived neurotrophic factor dysregulation, oxidative stress, and trace element imbalances further exacerbates these dysfunctions. **Conclusion.** According to our findings, the anatomy of the PFC appears to play a crucial role in the pathophysiology of ASD, given its involvement in executive function, emotional processing, and social cognition, suggesting a multifactorial pathophysiology that demands a multidimensional research approach.

Key Words: Anatomy ▪ Autism ▪ Brain Structure ▪ Synapse.

Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that emerges in early childhood and persists throughout an individual's lifetime. ASD is characterized by deficits in social interaction, communication difficulties, and the presence of restricted and repetitive behaviors. ASD significantly affects cognitive, emotional, social, and physical health, with an estimated prevalence of 1 in 36 children and a higher occurrence in males than in females (1). The prefrontal cortex (PFC) is anatomically located in front of the frontal lobe of the brain. This anatomical part of the brain, which governs high-level cognitive and social processes, has emerged as a critical region in ASD research. Impairments in activity-dependent neural signaling pathways and disruptions in trace element homeostasis within the PFC may underlie the cognitive and behavioral deficits characteristic of ASD (1).

This mini-review examines the existing literature on the structural and functional changes in the anatomy of the PFC associated with ASD, focusing on the roles of molecular signaling disruptions and trace element imbalances.

Methods

This literature review was developed through a structured search of academic publications published between 2010 and 2025. The databases searched were PubMed, Scopus, and Google Scholar. Relevant sources were identified using the search terms "Prefrontal Cortex Anatomy", "Autism Spectrum Disorder", "BDNF" (brain-derived neurotrophic

factor), "Oxidative Stress", "Neuroanatomy", and "Neurodevelopment". The reference lists of the selected articles were manually screened to identify further pertinent literature.

Inclusion Criteria: Studies were selected based on language (English), relevance to PFC morphological and anatomical alterations in ASD, and scientific rigor. Priority was given to research incorporating anatomical, neuroimaging, and neuropathological findings. *Exclusion Criteria:* Studies that examined connections between different brain regions or multiple disorders were excluded from the present review. Additionally, studies written in a language other than English or published before 2010 were excluded.

Data Extraction and Analysis

A data extraction form was used to extract data from eligible articles, which were reviewed simultaneously and independently by three reviewers (E.P., E.T., and M.T.). Disagreements were resolved through discussions among the reviewers and by team consensus.

Discussion

The etiology of ASD is multifaceted, involving a combination of genetic, epigenetic, and environmental factors that contribute to its heterogeneous presentation (1). Studies suggest that brain neuroanatomy, disruptions in neural circuits, synaptic plasticity, and neurotransmitter imbalances play significant roles in the pathophysiology of ASD (2). The PFC, a brain anatomical region responsible for executive functions, decision-making, and social cognition, has been identified as a critical area of interest in ASD research due to its involvement in higher-order cognitive processes (2).

BDNF Levels as a Potential Diagnostic Marker

Emerging human studies have investigated serum and plasma BDNF concentrations as possible biomarkers for ASD. Barbosa et al. (3) analyzed serum BDNF in 49 children with classical autism and 37 typically developing controls, finding that BDNF levels were statistically significantly elevated in ASD children ($P<0.000$). Their analysis included ROC modeling, suggesting moderate discriminatory ability between groups—though notable overlap and outliers cautioned against using BDNF alone as a diagnostic tool (3). A more recent study by Farmer et al. (4) emphasized that higher peripheral BDNF levels in ASD may largely reflect increased platelet counts rather than direct neural secretion. This finding highlights an important confounding factor when interpreting peripheral BDNF measurements and suggests that any biomarker development must account for platelet contributions (4). Taken together, while elevated peripheral BDNF in ASD is a reproducible finding, its standalone diagnostic utility remains uncertain without adjustments for biological confounders, such as platelets, age, and cognitive severity.

Structural and Functional Implications in the Prefrontal Cortex

Recent neuroimaging and histopathological studies have revealed abnormalities in the structure and function of the PFC in individuals with ASD, including altered neuronal connectivity, reduced dendritic spine density, and imbalances in excitatory and inhibitory neurotransmission (5). Additionally, recent studies have focused on the medial prefrontal cortex (mPFC), a crucial anatomical part of the "social brain" involved in social behaviors. These studies suggest that mPFC dysfunction may contribute to the changes in social behaviors observed in individuals with ASD (6). Furthermore, studies have indicated that mPFC dysfunction is associated with impaired emotional regulation and difficulties in interpreting social cues, further exacerbating the core symptoms of ASD (7, 8).

The functional connectivity between the PFC and other brain regions, such as the basal ganglia, thalamus, and cerebellum, plays a crucial role in the execution of complex motor, cognitive, and emotional functions. Disruptions in these connections have been associated with ASD symptoms,

highlighting the importance of large-scale brain network alterations in this disorder (8). Moreover, neuroanatomical and neurophysiological alterations in amygdala-PFC connectivity, particularly reduced connectivity between the amygdala and the right ventrolateral PFC during the processing of fearful faces, have been implicated in emotional dysregulation in ASD, further emphasizing the role of PFC dysfunction in socio-emotional impairments (9).

In a volumetric MRI study of children and adults with ASD, no significant differences in gross dorsolateral prefrontal cortex (DLPFC) volume were observed relative to typically developing controls, and volumetric measures did not correlate with executive task performance. These results suggest that executive dysfunctions may stem more from functional rather than structural gross abnormalities in this region (10).

In addition to the medial and ventrolateral PFC, there is strong evidence for the involvement of the DLPFC in ASD. For example, Courchesne et al. examined postmortem PFC tissue in children with ASD and neurotypical controls, focusing on the DLPFC (DL-PFC) and mesial PFC (M-PFC). They found that children with ASD had ~79% more neurons in the DL-PFC than controls, as well as an increased number in the M-PFC (~29%). These findings suggest that neuronal overpopulation in the DLPFC may play a role in the early brain overgrowth observed in ASD and that DLPFC abnormalities should be considered in models of structural PFC alterations (11). However, post-mortem examination has revealed microglial activation and altered neuron-microglia spatial organization in the DLPFC of individuals with autism. Microglia were found in closer proximity to neurons (e.g., 25–100 μm), potentially reflecting neuroinflammatory or homeostatic disruptions from early childhood onward (12).

In vivo proton magnetic resonance spectroscopy (MRS) investigations targeting adults with ASD have measured elevated gamma-aminobutyric acid (GABA)/water ratios in the left DLPFC, despite no significant difference in $GABA_A$ receptor density (13). This suggests that inhibitory neurotransmission in this region may be altered in ASD and could contribute to the functional atypicalities reported in executive control and inhibition tasks (13, 14). Functional magnetic resonance imaging (fMRI) studies, including those assessing temporal discounting, show reduced activation in both the right ventrolateral and dorsolateral PFC in adolescents and adults with ASD. Importantly, whereas typically developing individuals display increased activation with age in these regions, individuals with ASD exhibit attenuated functional maturation, which correlates with task performance and clinical indices, such as repetitive behaviors (15).

Together, these findings encourage a more integrative view: even in the absence of gross anatomical differences, the DLPFC exhibits functional under-activation, neurochemical alterations, and microglial–neuronal reorganization in ASD. These subtle changes likely underlie impairments in executive control, planning, inhibitory behavior, and cognitive flexibility. Including the DLPFC completes the anatomical and functional mapping of key PFC subregions implicated in ASD, offering a richer foundation for understanding the neural heterogeneity of the disorder.

Trace Element Dysregulation and Oxidative Stress

Neurochemical studies have also revealed significant alterations in the metabolic profile of the mPFC in individuals with ASD. Specifically, reduced levels of total N-acetylaspartate (tNAA) and total creatine, along with an increased Glx (mixed signal of glutamate and glutamine)/tNAA ratio, indicate underlying neurometabolic dysfunctions in the mPFC. These findings suggest potential disruptions in neuronal viability and energy metabolism, which may contribute to the cognitive and behavioral impairments observed in ASD (9). Additionally, emerging evidence suggests that oxidative stress and trace element imbalances, such as altered levels of copper (Cu), zinc (Zn), magnesium (Mg), and iron (Fe), may further influence neuronal function and exacerbate symptoms associated with ASD (7).

Recent studies have highlighted the role of glutathione (GSH), the primary antioxidant in the brain, in maintaining redox homeostasis within the PFC. Decreased GSH levels in individuals with ASD suggest impaired antioxidant defenses, leading to increased neuronal vulnerability and oxidative damage (16). Furthermore, trace elements are known to influence neurotransmitter systems—zinc plays a role in GABAergic transmission and synaptic inhibition, while iron is essential for dopamine synthesis, both of which are critical for emotional regulation and social behavior (17). Disruptions in these systems may intensify core ASD symptoms. In addition, trace element imbalances may affect epigenetic processes such as DNA methylation and histone modification, thereby altering the expression of genes linked to neurodevelopment (18). Notably, sex-based differences in oxidative stress responses and trace element metabolism may help explain the higher prevalence of ASD in males, underscoring the importance of individualized approaches in future research and therapy (19).

Key Morphological Findings or Alterations in Specific Prefrontal Cortex Regions

Morphological alterations of the PFC in ASD have been increasingly recognized as a central component of the neuroanatomical profile of the disorder. Beyond global volumetric changes, research emphasizes the importance of disentangling distinct morphological indices, such as cortical thickness, cortical surface area, and gray/white matter volumes. For example, Ecker et al. (20) reported that adults with ASD exhibit increased cortical thickness in the pars opercularis of the inferior frontal gyrus, coupled with reduced cortical surface area in regions such as the rostral middle frontal gyrus. These results suggest that atypical prefrontal development in ASD is not uniform but instead reflects a dynamic interplay between thickness and surface area that may follow distinct developmental trajectories. Importantly, such findings also highlight that volume-based measures alone may mask region-specific alterations that could be directly linked to behavioral phenotypes (20).

In addition, lifespan research indicates that these morphological differences are dynamic. Walsh et al. (21), for instance, demonstrated that reductions in hippocampal volume and increases in extracellular free-water are strongly associated with cognitive decline in older adults with ASD. While hippocampal changes were central to their study, the authors also underscored evidence of structural alterations in the prefrontal regions, particularly in the integrity of both gray and white matter, thereby suggesting that the PFC may undergo age-related modifications that interact with the clinical expression of ASD. This perspective underscores the necessity of adopting a developmental and longitudinal lens when examining prefrontal morphology (21).

It is worth noting, in comparison, that in adults with ASD, corresponding data reveal different trajectories: Braden and Riecken (22) demonstrated accelerated age-related cortical thinning, particularly in the pars opercularis of the frontal lobe, as well as in temporal, parietal, and occipital cortices. Although these adult findings do not provide detailed volumetric measures of gray and white matter, they highlight that cortical morphology in ASD is not static but evolves dynamically across the lifespan (22).

Finally, more recent neuroimaging data highlight that structural variability in the dorsolateral and orbitofrontal cortices is closely associated with symptom severity in ASD. Alterations in both gray and white matter organization within these regions—long recognized as critical for executive functioning, social cognition, and emotion regulation—point to the anatomical substrates of clinical heterogeneity across individuals (23). Taken together, these studies converge to demonstrate that the PFC in ASD is characterized by region-specific and age-dependent alterations in cortical thickness, surface area, and gray/white matter volumes.

Key Findings from Neuroimaging Studies of the Prefrontal Cortex in ASD

Neuroimaging research has provided substantial evidence of PFC dysfunction in individuals with

ASD. fMRI studies have revealed that individuals with ASD exhibit reduced activation in the right ventrolateral and dorsolateral PFC during tasks involving temporal discounting (15). Specifically, Murphy et al. (15) demonstrated that males with ASD had significantly lower brain activation in these regions than typically developing controls. This hypoactivation was associated with poorer task performance and suggests that deficits in PFC function may underlie decision-making impairments observed in ASD (15). In addition to functional abnormalities, structural differences in the PFC have been observed in ASD. Irimia et al. conducted a study using diffusion tensor imaging to assess white matter integrity in the PFC of individuals with ASD. Their findings indicated reduced fractional anisotropy in the left dorsolateral PFC, suggesting compromised white matter integrity in this region. These structural abnormalities may contribute to the functional deficits observed in the PFC and further support the notion of PFC dysfunction in ASD (24).

Future Research

Future investigations should prioritize longitudinal studies that integrate neuroimaging, molecular biology, and electrophysiology to establish causal links between PFC anatomical morphology and ASD symptomatology. Furthermore, exploring personalized therapeutic interventions, such as targeted neuromodulation and metabolic regulation strategies, may pave the way for more effective treatments. Addressing the heterogeneity of ASD through precision medicine approaches will be crucial for developing tailored interventions that enhance neurodevelopmental outcomes and improve the quality of life of individuals with ASD and their families. In summary, the anatomy of the PFC plays a critical role in the neurobiology of ASD, affecting cognitive, social, and emotional functions. Structural and functional abnormalities in the anatomy of this region, including disrupted connectivity, synaptic dysfunction, and neurochemical imbalances, contribute to the core symptoms of ASD. Studies additionally indicate that impairments in BDNF signaling and trace element homeostasis exacerbate these disruptions, further impacting neuronal plasticity and metabolic regulation. Given the complexity of ASD and the multifaceted involvement of PFC functions and anatomy, future research should focus on integrating neuroimaging, molecular, and electrophysiological approaches to develop targeted therapeutic strategies. Investigating the roles of oxidative stress, neuroinflammation, and genetic factors may provide deeper insights into the mechanisms underlying the pathophysiology of ASD. Additionally, personalized interventions based on an individual's neurobiological profile could enhance treatment efficacy, ultimately improving the quality of life of individuals with ASD.

Conclusion

The findings reviewed in this paper highlight the pivotal role of the PFC in the neurobiological mechanisms underlying ASD. Anatomic variations and structural and functional abnormalities within this region, including disruptions in neural connectivity, synaptic plasticity, and neurochemical balance, significantly contribute to the cognitive, social, and emotional deficits observed in ASD. The interplay between BDNF dysregulation, oxidative stress, and trace element imbalances further exacerbates these dysfunctions, suggesting a multifactorial pathophysiology that requires a multidimensional approach to research. In conclusion, the anatomy of the PFC plays a crucial role in the pathophysiology of ASD, given its involvement in executive function, emotional processing, and social cognition. Structural and functional disruptions, including impaired connectivity, synaptic alterations, anatomical variations, and neurochemical imbalances, highlight the complexity of the neurological basis of the disorder. Abnormal BDNF signaling and trace element dysregulation appear to aggravate these disturbances, indicating their potential as therapeutic targets. Advancing our understanding will require integrative approaches that combine neuroimaging, molecular biology, and electrophysiology to uncover

the underlying mechanisms. Ultimately, personalized, biology-driven treatment strategies may offer more effective interventions and significantly improve outcomes for individuals living with ASD.

What Is Already Known on This Topic:

ASD is a complex neurodevelopmental disorder with an increasing incidence and multiple impacts. Several hypotheses and mechanisms have been under investigation to identify the possible causes of ASD. The prefrontal cortex (PFC), an anatomical part of the brain that governs high-level cognitive and social processes, has emerged as a critical region in ASD research.

What This Study Adds:

After reviewing the available literature, this study clarifies that the anatomy of the PFC plays a crucial role in the pathophysiology of ASD and presents the possible mechanisms. Our findings could lead to further investigations and studies to improve outcomes for individuals living with ASD.

Authors' Contributions: Conception and design: ET and EP; Acquisition, analysis and interpretation of data: ET, AS and MT; Drafting the article: EP, MT and DC; Revising it critically for important intellectual content: DV and ET; Approved final version of the manuscript: DV, DC and TT.

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Review Article
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How *Histoplasma* Evades the Human Immune System

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Abstract
This review summarises the current knowledge of the interactions between *Histoplasma capsulatum* (Hc) and the human immune system, with particular emphasis on host immune responses and fungal immune evasion mechanisms that modulate disease pathogenesis and clinical outcomes. Histoplasmosis is a disease caused by Hc, a fungus found worldwide. Upon inhalation, complex interactions occur between the pathogen and the human immune system, primarily involving the recognition of fungal cell wall components. Both innate and adaptive immune responses are orchestrated to eliminate the fungus through a tightly regulated balance. However, Hc has evolved multiple strategies to evade host defences and establish infection. The clinical spectrum of histoplasmosis varies, ranging from isolated pulmonary involvement to disseminated disease, depending on host factors and pathogen characteristics. **Conclusion.** Overall, host-pathogen interactions between Hc and the human immune system play a central role in determining disease outcomes and represent key targets for improving preventive, diagnostic, and treatment strategies.

Key Words: Histoplasmosis ▪ Immune Evasion ▪ Pathogenesis ▪ Host-Pathogen Interaction.

Introduction

Histoplasmosis is a disease caused by fungi of the genus *Histoplasma*. Histoplasmosis in humans is classically caused by the fungi *Histoplasma capsulatum* (Hc) var. *capsulatum* and Hc var. *duboisii* (1). Histoplasmosis has been found worldwide, including in the Americas, Africa, Asia, Europe, and Australia, with the main endemic areas located in the Ohio and Mississippi River Valleys of the United States (2). In Latin America, positivity rates range from 37% to 90%, especially in Guatemala, Belize, Venezuela, and Brazil (3). The disease is increasingly recognised in Asia and Africa, with histoplasmin positivity rates of up to 86% in Asia and 0–35% in Africa (4, 5). In contrast, incidents in Europe and Australia are less frequent and primarily associated with travel-related cases, although limited endemic foci have been identified in Australia (3). Histoplasmosis can be fatal, especially in immunocompromised individuals, such as those with HIV/AIDS, and is characterised by systemic spread to various organs (6).

Misdiagnosis and co-infection occur due to similarities with other pulmonary diseases. Acute pulmonary histoplasmosis is frequently misdiagnosed as pneumonia, resulting in inappropriate antibiotic treatment that worsens outcomes (7, 8). Chronic pulmonary histoplasmosis may be misdiagnosed or co-infected with pulmonary tuberculosis because of the similarity in clinical and radiographic symptoms, such as cough, fever, weight loss, and chest X-ray findings of patchy pneumonic infiltrates, calcifications, cavities, and pulmonary nodules (7, 8). In a study of 213 patients with suspected pulmonary tuberculosis, 27 (12.7%) tested positive for Hc infection via antigen testing and/or PCR, indicating that histoplasmosis is relatively prevalent in this population. Of the 94 confirmed patients with TB, 7 (7.4%) had

histoplasmosis co-infection. However, 20 of the 119 patients who were not confirmed to have TB had histoplasmosis, suggesting that some cases may have been misdiagnosed as TB (9).

The high positive rate, frequent misdiagnosis, and co-infection in histoplasmosis cases in endemic areas indicate its significance, particularly for physicians and researchers in the prevention and control of infectious diseases. Histoplasmosis requires a comprehensive understanding of its pathogenesis for effective diagnosis, prevention, and treatment. This article outlines the fundamental aspects of the pathogenesis of Hc, including its cell wall, infection mechanisms, and host immune response. Hc develops mechanisms to evade or suppress the human immune response, allowing the infection to become chronic or spread to other organs. Hc has the potential to spread through the host's immune system. In addition, this study presents recent developments in Hc strain identification from diverse geographical regions, particularly Asia. These novel strains exhibit distinct genetic signatures and many virulence and host response variants, with potential implications for the severity of histoplasmosis and future treatments.

This review aims to examine the interactions between Hc and the human immune system, with a particular focus on fungal cell wall components, host immune responses, and immune evasion strategies, and to highlight their implications for prevention, diagnosis, and treatment.

Cell Wall Structure of *H. capsulatum*

The fungal cell wall is an essential structure for survival, morphology, and cell protection. Hc is a dimorphic fungal pathogen with a unique structure in its cell wall that plays a crucial role in its pathogenicity. Upon entering the host's body, Hc undergoes a phase transition to yeast, during which its cell wall contains essential components for communication with the environment and interaction with host immune cells, such as in the processes of phagocytosis and self-defence. The cell wall components of the yeast form of Hc are composed of various key elements, including carbohydrates, proteins, vesicles, lipids, and melanin (Figure 1) (10).

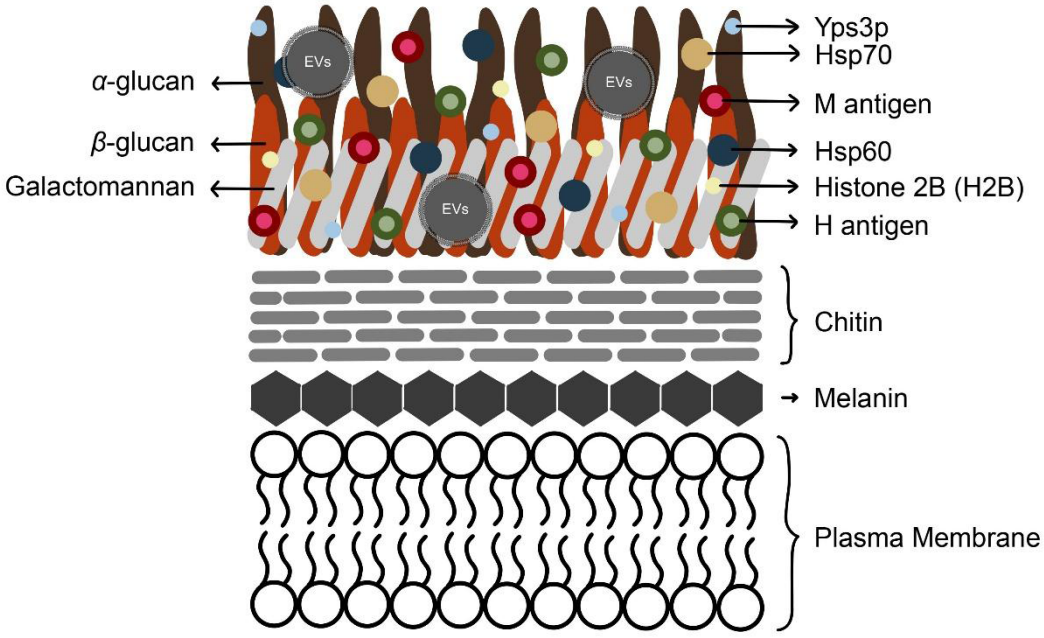

Figure 1. Cell wall schematic structure of *H. capsulatum* yeast (adapted and modified from Guimarães et al. (10)).

The carbohydrates in the yeast cell wall include chitin, glucan, galactomannan, lectin-like components, and mannoproteins. Chitin provides integrity and rigidity to the cell wall, providing structural protection against environmental pressures (10). Glucan is the primary carbohydrate component of the cell wall. They are D-glucose polymers that are connected by α- or β-glycosidic bonds. β-glucan, a major fungal polysaccharide also found in *Pneumocystis carinii* and *Saccharomyces cerevisiae,* has high antigenic properties that can bind to the Dectin-1 receptor on host macrophages and initiate an immune response (11). α-glucan has an important role in the virulence of several pathogenic fungi, such as *Aspergillus fumigatus*, *Blastomyces dermatitidis*, and *Paracoccidioides brasiliensis,* which masks yeast from immune recognition during morphogenesis (12, 13). Under normal conditions, only small amounts of α-glucan are found. However, after infecting the host, the amount of α-glucan increases significantly. This increase is important for helping the pathogen evade the host's immune response (immune evasion). The detailed mechanism and its contribution to successful infection are further discussed in the next section (10, 14).

In macrophage-based experimental models, galactomannan from the Hc cell wall triggers a direct response involving phagocytosis, synthesis of antimicrobial compounds, and release of cytokines, such as the pro-inflammatory cytokine IFN-γ and the regulatory cytokine IL-10. (15, 16). During phagocytosis, components such as lectin activate macrophages and agglutinate the host's erythrocytes (17). Mannoproteins are highly antigenic and induce the maturation and activation of dendritic cells, accompanied by the production of pro-inflammatory cytokines for host tissue adhesion (10).

The Hc cell wall contains protein molecules, such as heat shock protein (Hsp), M antigen, H antigen, histone 2B (H2B), and Yps3p. Heat shock proteins respond to extreme conditions, especially in the human body (10, 18). Several types of Hsp have been identified in the cell wall of Hc, such as Hsp of 60 kDa (Hsp60) and Hsp of 70 kDa (Hsp70). Hsp60 is a major ligand attached to the CR3 receptor on macrophages that triggers phagocytosis. Its expression depends on the response to temperature stress, peaking at 34-37°C. The role of Hsp60 is to support cell wall changes and increase energy gain. Hsp70 expression increases during the mycelial-to-yeast phase transition and peaks at 37°C (10).

M and H antigens are glycoproteins found on the wall of Hc that are homologous to catalase and β-glucosidase (19, 20). Antigen M is the catalase possessed by Hc, both within the cell wall and secreted outside the cell. This catalase is classified as an antigen based on its amino acid sequence and reactivity with monoclonal antibodies (21). There are three catalases in Hc: catalase B (CatB) and catalase A (CatA), secreted outside the fungal cell, and catalase P (CatP), which is secreted inside the cell. CatA is primarily produced during the mycelial phase, whereas CatB and CatP are produced during the yeast and mycelial phases. These three catalases protect Hc from oxidative stress and promote survival in host cells. The H antigen is a β-glucosidase homologue that helps in the breakdown of carbohydrate substrates from the environment to produce glucose as an energy source and cell wall modulation. Both antigens are secreted and react with the patient's serum (20).

The Hc cell wall also contains H2B, which is speculated to be a protein used in cell signalling that modulates the immune response of the fungus (10). Yps3p can bind to chitin and is a virulence factor that can increase the spread of phagocytic cells in tissues. These proteins can be used to characterise Hc with distinctive molecules (10).

The cell wall of Hc yeast produces extracellular vesicles (EVs) that contain various lipids, carbohydrates, proteins, pigments, and nucleic acids. These vesicles can function as "virulence bags" because they concentrate virulence factors that trigger stress responses and pathogenesis, such as urease, phosphatase, catalase, and laccase (22, 23). Additionally, proteins extracted from Hc vesicles react with the immune serum of patients with histoplasmosis, indicating that these vesicles can modulate the immune response (23).

Hc can synthesise melanin in its cell wall, a negatively charged hydrophobic pigment with a high molecular weight formed through the oxidative polymerisation of phenolic and/or indolic compounds. Melanin in Hc reduces susceptibility to host defence mechanisms and antifungal drugs, such as amphotericin B and caspofungin (10, 24, 25). Melanin binds to antifungal molecules and prevents the drug from interacting with ergosterol on the cell membrane, thus localising the antifungal compound in the extracellular space. This pigment is also commonly found in various human pathogenic fungi, including *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Candida albicans*, and *Sporothrix schenckii*, underscoring its critical role in fungal survival and virulence (26). Given its role in virulence and antifungal resistance, melanin could be a potential therapeutic target in histoplasmosis, as inhibition of its biosynthetic pathways may enhance the efficacy of histoplasmosis therapy.

Host Immune Response to *H. capsulatum* Infection

Hc microconidia and hyphal fragments enter the host via inhalation and convert into the yeast form in response to body temperature as they reach the lung tissue (Figure 2). The first line of defence is mucociliary clearance, where mucus traps inhaled particles and cilia expel them; however, Hc can evade this due to its small size. In the alveoli, it faces surfactant proteins, particularly SP-A and SP-D, which opsonise pathogens and enhance phagocytosis by macrophages and neutrophils, as well as exert fungicidal effects by disrupting fungal cell walls. To survive, the fungus hides within macrophages and escapes surfactant-mediated defences (2). Hc also interacts with various cell responses of the innate immune system and later adaptive immune response, as summarised in Table 1.

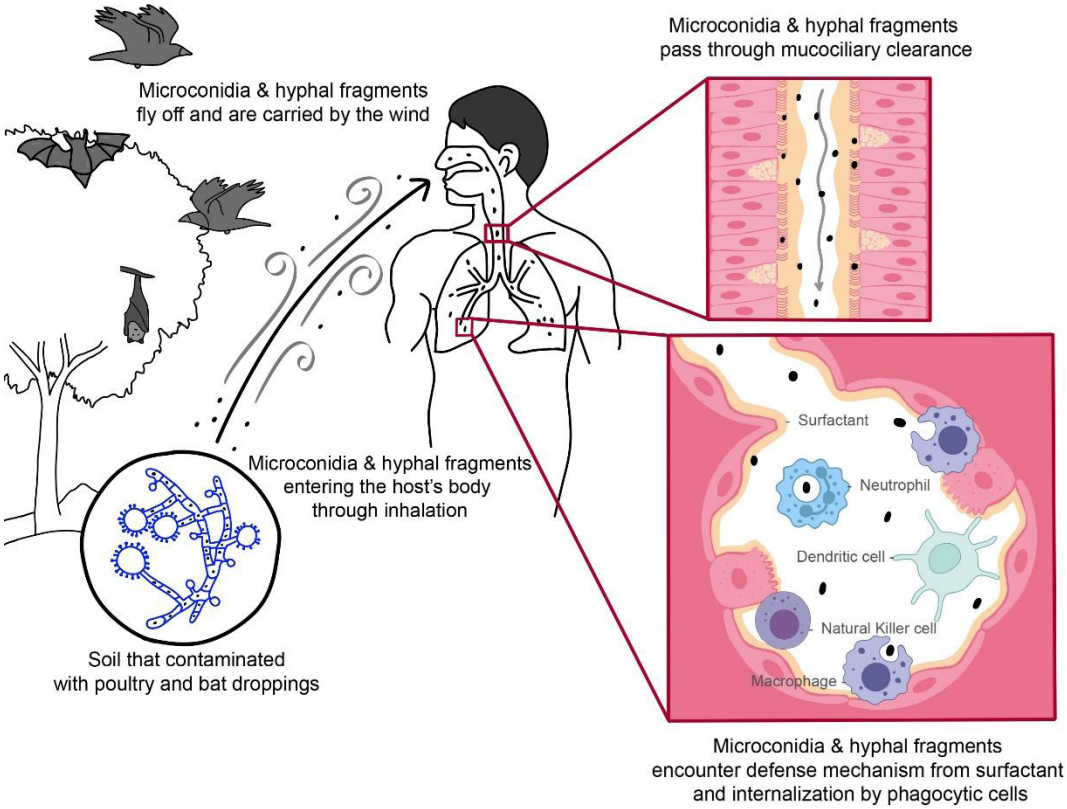

Figure 2. *H. capsulatum* infection in the human body (adapted and modified from Mittal et al. (2)).

Table 1. Immune Response to *H. capsulatum*

Host property	Mechanism
Mucociliary Clearance (2)	The respiratory epithelium produces mucus, which traps, and cilia push microorganisms towards the pharynx to be swallowed or expelled
Surfactant protein (2)	SP-A & SP-D opsonise microbes, thereby increasing phagocytosis by macrophages and neutrophils
	Fungicidal properties by disrupting the fungal cell wall, therefore increasing the permeability
Macrophage (27, 31–33)	CR3 & Hsp60 interaction causing internalisation of Hc
	Dectin-1 & β-glucan interaction causes the release of pro-inflammatory cytokines: IL-6 and TNF-α
	TLR2 & Yps3p interaction causes activation of the NF-κB cascade, which activates the adaptive immune response via T cells
	Dectin-1 & galactomannan interaction causes the release of IFN-γ and IL-10
	Reducing copper availability in the phagosome
	Iron limitation occurs as activated macrophages increase iron binding to transferrin, reducing free plasma iron levels
	GM-CSF induces metallothionein expression, leading to zinc sequestration
Dendritic Cell (34–36)	VLA-5 & CypA interaction causing: Phagocytosis of the fungi by DCs. Inhibit the ability of Hc to control phagolysosome formation Intracellular oxidation through the release of hydrolases
	Dectin-1 & β-glucans also Dectin-2 & α-mannans interaction causing activation of NLRP3 inflammasome protein and further inducing dendritic cells to secrete IL-1β and IL-18, which will mobilise DCs to lymph nodes for antigen presentation & priming of naïve T cells
	TLR7 and TLR9 in dendritic cells detect fungal nucleic acids, promote IFN-1 production
Neutrophil (37–40)	CR1, CR3 & C3B opsonin interaction causes an increase in phagocytosis
	Release of NET to trap & kill Hc
NK Cell (41)	Activating T cells for further adaptive immune response
CD4+ T Cells (41)	IL-12 drives the differentiation of naïve T-cells into Th1 cells and stimulates IFN-γ and TNF-α production by Th1 cells, which activates more macrophages, resulting in oxidative burst
	Th2 cells produce cytokines such as IL-4 & IL-21, which drives immunoglobulin class switching
	Th17 cells enhance fungal clearance by influx of inflammatory cells
Humoral Immune Response (45, 46)	Antibodies towards Histone 2B on fungal walls lower fungal burdens, reduce pulmonary inflammation, and impair Hc's ability to regulate intraphagosomal pH within macrophages
	Antibodies directed against Hsp60 promote agglutination, which in turn hinders the spread of the fungus
	Antibodies targeting the M antigen enhance macrophage-mediated phagocytosis of the yeast and boost the host cell's ability to kill the pathogen

SP-A=Surfactant protein A; SP-D=Surfactant protein D; CR1=Complement receptor 31; CR3=Complement receptor 3; Hsp60=Heat shock protein 60: TNF-α=Tumour necrosis factor alpha; TLR2=Toll-like receptor 2; Yps3p=Yeast phase-specific protein, NF-κB=Nuclear factor-kappa B; IFN-γ=Interferon-gamma; VLA-5=Very late antigen 5; CypA=Cyclophilin A; NLRP3=Nucleotide-binding domain; Leucine-rich–containing family; Pyrin domain–containing-3; NET=Neutrophil extracellular traps.

Macrophages

Macrophages are key in host defence against Hc, internalising the fungus via surface receptors such as CR3 without requiring opsonisation, a crucial mechanism in the low-opsonin environment of the lungs (27). The ligand that interacts with macrophage receptors is Hsp60, which is expressed on the surface of yeast Hc. Heat shock proteins generally play a role in protein folding but also have immunogenic properties in several pathogens, such as *Borrelia burgdorferi, L. pneumophila, Chlamydia trachomatis,* and many others, indicating that this protein is a potent antigen that triggers a host immune response (28). In vitro studies using cultured immune cells have demonstrated that macrophages recognise Hc through several receptors: CR3 binding to Hsp60 mediates fungal internalisation; Dectin-1 binding to *β*-glucan induces IL-6 and TNF-*α* release; and

TLR2 interaction with Yps3p activates the NF-κB cascade, triggering T-cell responses (29). However, Hc is known for its ability to survive and even multiply inside the macrophage through several mechanisms. These mechanisms are further discussed in the next section (30).

Macrophages also function as innate immune cells that recognise the structural components of the Hc cell wall, including galactomannan. A study using peritoneal macrophages extracted from rodents demonstrated that galactomannan is recognised by the C-type lectin receptor Dectin-1, leading to fungal phagocytosis and the induction of IL-10 and IFN-γ, but not TNF-α. IFN-γ subsequently enhances macrophage activation, resulting in reduced intracellular Hc survival. In contrast, IL-10 exerts anti-inflammatory effects that may limit tissue damage by dampening excessive pro-inflammatory responses (31).

Another role of macrophages is to reduce the availability of trace metals within macrophages. A study has shown that activation of macrophages by IFN-γ changes the phagosomal environment to a copper-, iron-, and zinc-deficient environment. Iron, zinc, and copper are essential nutrients for Hc. The limitation of iron is due to a host mechanism that increases the affinity of iron-transferrin binding, thereby reducing free plasma iron concentration (32). An in vitro macrophage experiment reported that zinc sequestration is triggered by granulocyte-macrophage colony-stimulating factor (GM-CSF), which causes the expression of cytoplasmic zinc-binding metallothioneins and redistributes zinc from intraphagosomal Hc yeast to the Golgi compartments. When their availability is restricted, intracellular fungal growth is inhibited. However, Hc can overcome copper deficiency via the Ctr3 transporter mechanism, which is discussed in the following section (33).

Dendritic Cell

The interaction between Hc and DC is mediated by very late antigen 5 (VLA-5), Dectin-1, and Dectin-2 receptors. Using a human dendritic cell culture system, a study demonstrated that the interaction of VLA-5 with its ligand CypA causes phagocytosis and triggers further intracellular signalling to inhibit the ability of Hc to control phagolysosome formation (34). The eradication of Hc in DCs is mainly due to oxidative burst reactions mediated by the release of hydrolases instead of NO. Other DCs receptors, Dectin-1 and Dectin-2, could recognise β-glucans and α-mannans of the Hc cell wall, respectively. This interaction activates the NLRP3 inflammasome protein and further induces DCs to secrete IL-1β and IL-18, which play a role in the mobilisation of DCs to the lymph nodes for antigen presentation and priming of naïve T cells (35). Additionally, dendritic cells have intracellular receptors that, when activated, result in the production of type 1 interferon (IFN-1) (36).

Neutrophil

Neutrophils can phagocytose and release neutrophil extracellular traps (NETs) to eradicate Hc. Neutrophils can phagocytose pathogens through opsonisation mechanisms, such as via CR1, CR3, and FcγRIII (CD16) recognition of C3b opsonins. Neutrophils can also act via opsonin-independent mechanisms, although the specific receptors and pathways involved are not yet fully understood (37). However, this mechanism is unlikely to be the primary cause of the fungistatic effect of neutrophils. This is evidenced by the fact that, in a study with isolated human neutrophils, inhibition of phagocytosis using cytochalasin D still results in the inhibition of Hc (38).

The primary mechanism by which neutrophils exert fungistatic and even fungicidal effects is through the release of neutrophil extracellular traps (NETs), which are a network of extracellular strings consisting of DNA, histones, and antimicrobial proteins that trap pathogenic microorganisms (39). The trapped pathogens are then destroyed by the antimicrobial proteins contained in the neutrophil azurophilic granules. An *in vitro* study using purified neutrophil granules on Hc reported that bactericidal permeability-increasing proteins (BPI) contained in neutrophil azurophilic granules were able to inhibit the growth of

this fungus. In addition to BPI, NETs also contain cathepsin G and defensin proteins, which have been shown to effectively inhibit the growth of Hc yeasts, although the exact mechanisms involved remain unclear (40).

Natural Killer Cell

Macrophages that phagocytose Hc release the cytokine IL-12, which activates NK cells to produce IFN-γ, helping to control the Hc infection. In an in vivo study with mice infected with Hc and treated with IL-12, the investigators found that IL-12 treatment significantly reduced mortality, increased IFN-γ production, decreased fungal burden in spleen cells, and that the protective effect of IL-12 was dependent on IFN-γ. However, its significant role in the elimination of the fungus from the host Hc has yet to be clarified (1).

T Cell

Dendritic cells (DCs) and macrophages function as antigen-presenting cells (APCs) that activate naïve T-cells through antigen presentation in the context of MHC class II molecules. This interaction is enhanced by the production of the key cytokine IL-12 by DCs and macrophages. IL-12 drives the differentiation of naïve T-cells into Th1 cells and stimulates IFN-γ and TNF-α production by Th1 cells, which subsequently strengthens the cellular immune response against intracellular pathogens such as Hc by activating more macrophages, resulting in an oxidative burst (42). A study in mice injected with anti-IFN-γ antibodies and IFN-γ gene knockout mice reported that they were more susceptible to lethal infection than the control group, showing the crucial role of IFN-γ in the host's innate defence against systemic Hc infection (43).

Differentiation into Th2 cells is driven by IL-4. Cytokines with immunosuppressive effects, such as IL-4 and IL-10, can hinder the immune response against Hc. An in vivo study in mice has shown that their combined activity suppresses the development of IFN-γ–producing cells, which are critical for fungal clearance. A longitudinal study found that as IL-4 and IL-10 levels declined, the number of IFN-γ-producing cells induced by Hc increased significantly, leading to improved fungal elimination. Additionally, IL-4 can support the survival of intracellular pathogens by reducing nitric oxide (NO) production and increasing the levels of intracellular metal concentrations in macrophages. This IL-4–mediated zinc, calcium, and iron regulation has been shown to promote fungal replication, with increased metal levels partially restoring yeast growth (44).

Granulomas, formed by macrophages and lymphocytes during Hc infection, restrict fungal replication and prevent systemic spread. In murine models, infiltration begins by day 5, granulomas form by day 7, peak at day 10, and eventually eliminate most fungi, although some latent yeast may persist and reactivate under immunosuppression (45). Studies in mice have shown that reactivation occurs in animals with depleted CD4 and CD8 T cells (44).

In addition to the Th1 immune response, an experimental study conducted in mice has shown that Th17 also plays a minor role in host defence against Hc. Naïve T cells differentiated to Th17 in response to cytokines such as IL-6 and IL-23. Th17-cytokine, IL-17, facilitates further recruitment of inflammatory cells, such as macrophages and neutrophils, to the lungs during infection (44). Wüthrich et al. implied that the fungal load in vaccinated mice without IL-17 receptor was higher than that in vaccinated wild-type controls, revealing its significance in fungal infection (46).

Humoral Immune Response

In general, B cell activation occurs through two pathways: T-independent and T-dependent activation. T-independent activation involves non-protein antigens that directly stimulate B cells through BCR cross-linking and TLR/complement interactions, producing short-lived IgM without memory cells. T-dependent activation requires protein antigens presented by B cells via MHC II to helper T cells, with Th2 cytokines, such as IL-4 and IL-21,

driving class switching to IgG, IgA, and IgE. While T-independent responses provide rapid but temporary protection, T-dependent responses create durable and adaptable immunity. Although the immune response to Hc is primarily driven by cellular immunity, the humoral response also plays a role. An experiment in mice showed that depletion of $CD4^+$ and $CD8^+$ T cells in B-lymphocyte knockout mice resulted in significantly higher fungal burdens in organs than T cell depletion in wild-type mice in a model of secondary histoplasmosis (47).

The cell wall proteins of Hc (melanin, H2B, Hsp60, and M antigen) stimulate antibody production, with IgM, IgA, and IgG peaking by day 21 in murine models. Monoclonal antibodies against H2B and Hsp60 reduce fungal growth, impair survival mechanisms, promote agglutination, and enhance macrophage-mediated phagocytosis, thereby improving host defence (48).

Immune Evasion and Yeast Survivability Factors

Dimorphism is a crucial strategy of Hc to evade the immune system (Figure 3). The transition to its yeast form is primarily triggered by an increase in temperature to 37°C. Stepwise genetic analyses that used random insertional mutagenesis was conducted to identify mutants unable to undergo the mycelial to-yeast transition at 37°C. Following mapping and characterisation of the disrupted loci revealed Drk1, which codes for a hybrid histidine kinase, a protein that integrates environmental sensing and signal transduction. Mutants with Drk1-deficient strains were still in the mycelial phase after being exposed to host temperature. These results established that Drk1 is a crucial signal transducer that mediates temperature-induced pathogenic yeast form (49). These findings were primarily derived from in vitro cell culture-based experiments. Additionally, yeast-phase conversion in synthetic media requires exogenous cysteine, as demonstrated by early chemical complementation experiments showing that cysteine supplementation restores respiration even when the respiratory pathway is inhibited and that the role of cysteine cannot be entirely replaced (50-52). Cysteine, with its sulfhydryl (-SH) group, functions as a reducing agent that reactivates mitochondrial respiration, which is crucial for meeting metabolic demands during the yeast phase. This reactivation is necessary to complete the morphological transition and the pathogenicity of Hc (53, 54).

Furthermore, four transcription factors, Ryp1, Ryp2, Ryp3, and Ryp4, play a role in this differentiation switch by forming an interdependent loop. The binding of multiple Ryp factors to their respective promoter regions, such as Sod3, CatB, CatP, and Yps3, affects the virulence of Hc. These regulatory interactions have primarily been characterised using in vitro cell culture–based models. In addition, Vea1 is also important for yeast-phase morphogenesis in Hc. Mycelial phase factors, such as Wet1, are suppressed at 37°C to prevent hyphal growth (55).

Once Hc has established its yeast phase, it must overcome the unfavourable environments posed by the host's immune system (Table 2). Two of the most well-established mechanisms by which Hc avoids immune system detection are the production of α-glucan and Eng1. Synthesis of α-glucan occurs only during the yeast phase of Hc. The production of this polysaccharide covers the outer layer of the yeast cell wall and covers β-glucan (56). This mechanism is essential for preventing the interaction between Dectin-1 and β-glucan, avoiding the detection of Hc by macrophages, and preventing the production of pro-inflammatory cytokines. In contrast, Eng1 is a protein of Hc yeast that plays a role in hydrolysing β-glucan, thereby shearing off the exposed β-glucan. These immune evasion strategies have been predominantly characterised using in vitro cell culture–based systems, including fungal cultures and host immune cell interaction assays. Similar to α-glucan, this process is proposed to minimise detection by the host immune system (57).

Hc yeast counteracts host-derived oxidative stress by producing multiple antioxidant enzymes, as demonstrated in both in vitro phagocyte infection models and in vivo murine studies. In macrophage and polymorphonuclear neutrophil (PMN)

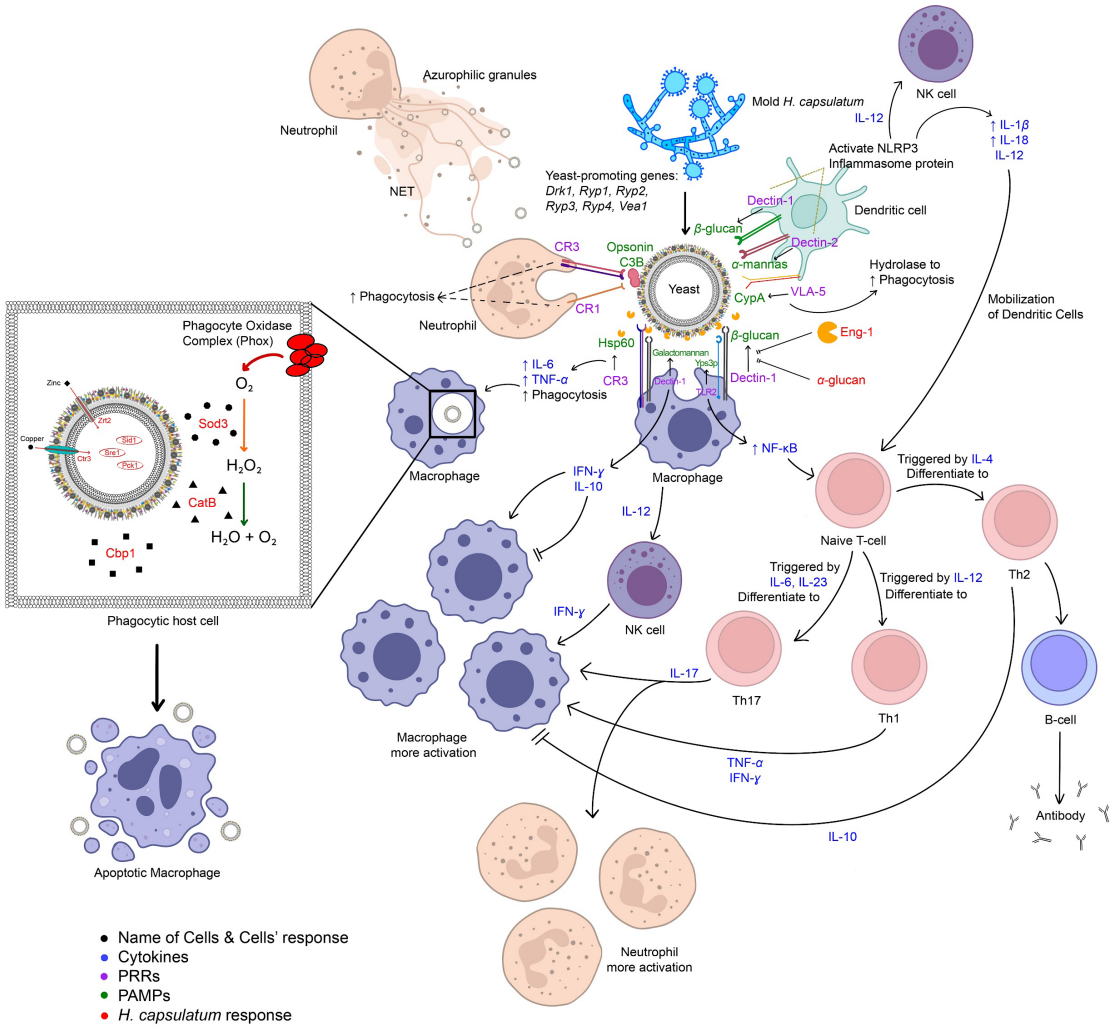

Figure 3. Immune response to *H. capsulatum*. Conceptual framework based on published studies summarised in Tables 1 and 2.

infection assays, Hc yeast secretes an extracellular Cu/Zn-type superoxide dismutase, Sod3, which detoxifies extracellular superoxide generated by host phagocytes, thereby enhancing fungal survival under oxidative stress conditions. (30). Hc yeast also produces CatB and CatP to counteract host cell defence, which function similarly to Sod3. CatB primarily protects against extracellular reactive oxygen species, whereas CatP acts intracellularly. Loss of CatB significantly reduces yeast survival in PMNs and attenuates virulence in mice, whereas deletion of CatP alone has minimal impact. These findings indicate that Hc pathogenesis largely depends on the neutralisation of exogenous oxidative stress (58).

The glutathione system has been shown to participate in stress adaptation and virulence in pathogenic fungi, as demonstrated primarily through in vitro stress-response assays and targeted gene disruption studies, and supported by in vivo infection models. In eukaryotes, glutathione serves as an important metabolite and an essential participant in the protection against oxidative damage. Hc possesses GSH1 and GSH2 genes, which produce γ-glutamylcysteine synthetase and glutathione synthetase enzymes, respectively, which contribute to this system. An intact

Table 2. *H. capsulatum* Immune Evasion and Yeast Survivability Factors

Detection evasion	
Eng1 (54)	Trims excess β-glucan to further minimise host immune system detection
α-glucan (55)	Covers β-glucan layer to avoid detection of Dectin-1
The host immune system is hampering	
Sod3 (53)	Dismutes extracellular superoxide to increase yeast survivability
CatB (57)	Responsible for extracellular catalase activity to increase yeast survivability
CatP (57)	Responsible for intracellular catalase activity to further increase yeast survivability
Gsh1 & Gsh2 (58)	Plays a role in the glutathione system, protecting yeast from oxidative damage
NIT50 and other (59)	Hypoxia-responsive genes increase survivability in hypoxic conditions
Macrophage lysis	
Cbp1 (68)	Lyses macrophage
Yeast survival and proliferation factors	
Ctr3 (32)	Forms a copper transporter in a low-copper environment
Srb1 (59)	Vital for recovering from hypoxic conditions
Sre1 (61)	Forms iron-scavenging siderophores in an iron-limited environment
Zrt2 (62)	Forms a zinc transporter in a zinc-restricted environment
Pck1 and other (65)	Gluconeogenesis for carbon sources
Trp5 (66)	Tryptophan biosynthesis, important for yeast proliferation
Rib2 and other (67)	Vitamin synthesis, important for yeast proliferation

Eng1=Endo-1,3 (4)-beta-glucanase 1, Sod1=Superoxide dismutase 1; CatB=Catalase B; CatP=Catalase P; Gsh1=γ-glutamylcysteine synthetase enzyme; Gsh2=glutathione synthetase enzyme; NIT50=nitrosative stress induced transcription 50; Cbp1=Calcium binding protein 1; Srb1=Sterol regulatory element binding protein 1; Ctr3=Copper uptake protein 1; Sre1=Sterol regulatory element 1; Sid1=Enzyme for siderophore production; Zrt2=Zinc uptake protein 2; Pck1=Phosphoenolpyruvate carboxykinase 1; Trp5=Tryptophan synthase 5; Rib2=Riboflavin synthase.

glutathione system is necessary for successful host adaptation and pathogenicity, according to in vitro experimental analyses that revealed disruption of this pathway reduces virulence in animal models and fungal survival under oxidative stress (59).

Hc could also increase its survival by counteracting the host's adaptive immune response. In murine models, the fungus responds to hypoxia due to inflammation and granuloma formation by producing the Srb1 transcription factor. Srb1 influences the expression of several hypoxia-responsive genes (HRG), such as nitrosative stress-induced transcription 50 (NIT50), an ABC transporter, NADP/FAD oxidoreductase, and an RSP/GEF, which help in survival under hypoxic conditions. Additionally, silencing Srb1 causes hypersensitivity to itraconazole (60).

Hc has several mechanisms to overcome sequestration and the reduction of essential metals. The fungus secretes iron-scavenging siderophores, which are regulated by the GATA transcription factor (Sre1) (61) and require L-ornithine-N^5-monooxygenase (Sid1) in response to low iron levels inside the macrophage, as proven by Hwang et al. and Hilty et al., respectively, using high-iron and low-iron media (61, 62). These siderophores are pivotal in binding iron for further yeast proliferation, while phagosome iron levels decline (62). In the case of zinc sequestration, Hc responds by increasing the transcription of Zrt2, forming a zinc transporter with both high and low affinity (63). Dade found that Zrt2 was crucial on the fifth day post-infection in mice because zinc supply becomes more restricted due to the previously mentioned GM-CSF activation (64). As pathogenesis progresses, copper is restricted by the activation of the host macrophage by IFN-γ. Hc possesses Ctr3, which functions as a copper importer to support yeast growth in low-copper environments. This importer is formed by CTR3 gene expression. It is known that there

are two other genes, CTR1 and CTR2, expressed by Hc yeast. According to Shen et al., all of these genes are expressed equally during the mycelial phase of Hc; however, the expression of CTR3 can reach 10-fold higher in a low-copper environment during the yeast phase, which was proven using a 3M medium containing low or high $CuSO_4$ (33). CTR3 expression is regulated by Mac1, a copper-dependent transcription factor activated in a low-copper environment. A study by Ray et al., utilising both *in vitro* and *in vivo* (murine models) methods, showed that Mac1 is also responsible for Hc virulence, facing the host's adaptive immune response, metal homeostasis, and ROS detoxification (65).

The intracellular environment is characterised by limited carbon sources, requiring Hc yeast to rely on alternative carbon sources for energy. Gluconeogenesis is the most critical pathway for carbon acquisition. Moreover, the primary source of carbon utilised by Hc yeast is derived from amino acids. Shen et al. used an *in vitro* method, which demonstrated that Hc can metabolise single amino acids or short peptides digested by proteinase K or cathepsin D, but not intact proteins. Notably, disruption of phosphoenolpyruvate carboxykinase 1 (Pck1) and fructose-1,6-bisphosphatase (Fbp1) impairs the ability of yeast to utilise gluconeogenic substrates, thereby compromising its virulence. Moreover, this study also found that intracellular Hc did not utilise hexose or fatty acids as carbon sources (66).

Among the various types of amino acids, tryptophan is indispensable for Hc growth and full virulence. Shen et al. evaluated the importance of aromatic amino acid biosynthesis for Hc proliferation. By silencing PHA2, TYR1, and TRP5, Hc lost the ability to encode prephenate dehydratase, prephenate dehydrogenase, and tryptophan synthase, respectively. This depletion also leads to auxotrophy for phenylalanine in Pha2-deficient samples, tyrosine in Tyr1-deficient samples, and tryptophan in Trp5-deficient samples. The study experimented on these auxotrophs by infecting a macrophage population in which Pha2-deficient and Tyr1-deficient Hc were able to kill 80% of the macrophage population, whereas Trp5-deficient Hc were only able to wipe out 20% (67).

Vitamins also contribute to the proliferation of Hc yeast. A study showed that Hc yeast can independently synthesise most vitamins. However, not all synthesised vitamins are essential for proliferation. Garfoot et al. proved that vitamins, such as riboflavin (B2), pantothenate (B5), and biotin (B7), are crucial for Hc yeast proliferation. Silencing their respective genes (RIB2, PAN6, and BIO2) resulted in a severe decrease in the replication rate, fungal burden, and macrophage lysis ability of Hc in mice (68).

Hc can also induce macrophage lysis. The fungus produces Cbp1 protein within macrophages and is fully active in the yeast form (69). Isaac et al. showed that Cbp1 is essential for macrophage lysis and could also prolong yeast cell proliferation in macrophages (69). English et al. found that without Cbp1, Hc yeast cannot trigger host cell death, even with an increasingly high intracellular fungal burden. The same study also proved that Cbp1 affects the virulence of Hc yeast in murine models of histoplasmosis. Subjects infected with lethal doses of wild-type Hc died in 9 days, while subjects with Hc mutants without Cbp1 survived (70).

English et al. also proved that Hc yeast expressing Cbp1 caused macrophages to activate the integrated stress response (ISR) by measuring the level of activating transcription factor 4 (ATF4) in bone marrow-derived macrophages (BMDMs). With an increase in ATF4 levels, the transcription factor C/EBP homologous protein (CHOP) and tribbles pseudokinase 3 (TRIB3) are upregulated (71). These two transcription factors are pro-apoptotic, which explains how Cbp1 can cause macrophage apoptosis (70).

Hc growth also increases in a relatively high amount of CO_2. As mentioned previously, host macrophages produce ROS in an attempt to eradicate endocytosed pathogens. To secrete ROS, the pentose phosphate pathway is activated to produce NADPH, a key component of ROS production, which serves as a reductant in the NADPH oxidase reaction. Subsequently, this pathway excretes CO_2

as a byproduct, thereby increasing the CO2 content within macrophages. Shen et al. discovered that Hc could utilise this increase in CO_2, contributing to its virulence. Hc growth and its antifungal resistance, especially towards itraconazole and caspofungin, were enhanced on solid *Histoplasma*-macrophage medium under 5% CO_2 compared to ambient air (72).

Pathogenesis and Clinical Relevance

The host immune status is a critical determinant of the severity of histoplasmosis. In experimental murine models, depletion of CD4+ T cells, alone or in combination with CD8+ T cell depletion, transforms a self-limited pulmonary infection into a progressive and fatal course characterised by higher fungal burdens and impaired inflammatory responses, recapitulating the features of histoplasmosis in immunocompromised patients (73). These findings parallel clinical observations in individuals with advanced HIV infection and low CD4+ counts, in whom loss of cellular immunity permits the dissemination of Hc beyond the lungs and failure of granulomas to contain the fungus (74). Likewise, pharmacologic immunosuppression disrupts host defence, such as TNF-α inhibitors, which compromise granuloma formation and maintenance and are associated with disseminated histoplasmosis. Long-term corticosteroid therapy has also been associated with more severe and progressive disease due to the suppression of multiple cell-mediated pathways (75).

Disseminated disease generally reflects a high fungal burden; therefore, diagnostic strategies often focus on tests that detect fungal antigens or fungal load rather than host antibody responses. The culture and histopathology of clinical specimens, including blood, bone marrow, respiratory specimens, and tissue biopsies, provide definitive evidence of histoplasmosis. In disseminated disease, these methods have a higher diagnostic yield due to the increased fungal burden, although the results may be delayed, and the sensitivity can vary by specimen type. The detection of Hc galactomannan antigen in urine or serum is highly sensitive for disseminated disease and useful for early diagnosis and monitoring of response to therapy (76, 77). The 100 kDa protein (Hcp100), which has shown promise as a diagnostic antigen with reduced cross-reactivity against other pathogenic fungi, is one of several alternative antigen candidates with improved specificity that have begun to be investigated (74). In contrast, antibody testing against H and M antigens (immunodiffusion test) and the crude yeast/mycelial phase antigen (complement-fixation test) play supportive roles but may be limited in immunosuppressed individuals with impaired humoral responses. Molecular methods, such as PCR, have been used and have shown high sensitivity and specificity, particularly in disseminated disease. However, implementation remains limited due to the lack of a standardised protocol and validated targets (78).

In addition to existing diagnostic tests, there remains a need for methods capable of detecting latent histoplasmosis while retaining sensitivity to active infection. Recent advances have explored diagnostic approaches that leverage knowledge of Hc-specific cellular immunity, including the interferon-gamma release assay (IGRA). This assay detects Hc infection by measuring IFN-γ released from sensitised T cells following stimulation with Hc antigens. Datta et al. evaluated this method in individuals with suspected or confirmed histoplasmosis as well as in healthy controls, reporting a specificity of 100% and a sensitivity of 77.2%, with the ability to identify healthy individuals with evidence of latent Hc infection. Overall, these findings suggest that IGRA represents a promising adjunctive diagnostic tool for Hc infection (79). However, further development and validation are required before routine clinical implementation.

Therapeutic decisions are guided by disease severity and host immune competence. In immunocompetent hosts with mild acute pulmonary histoplasmosis, effective cellular immunity often leads to spontaneous resolution, and antifungal therapy may not be required. In moderate or chronic pulmonary disease, treatment with oral azoles, such as itraconazole, is recommended to inhibit fungal growth (fungistatic) while the host

immune system clears the infection. For severe or disseminated histoplasmosis, especially in immunosuppressed patients, initial therapy with a fungicidal agent, such as liposomal amphotericin B, is recommended, followed by step-down therapy with itraconazole. Immune reconstitution, for example, with antiretroviral therapy in HIV-infected patients, is an essential component of treatment to restore effective cellular immunity (80, 81).

Current research on vaccines and immunotherapies for Hc remains in the preclinical stage, with no licensed human vaccines available. Several cell wall components, such as H antigen, Hsp60, and Hsp70, have been studied for their potential to induce protective immunity. These components have been found to elicit cell-mediated immune response; however, only Hsp60 induce protection against intranasal inoculation of Hc yeast cells in a murine model. Deepe et al. demonstrated protective immunity conferred by a crude alkaline extract of Hc packaged in glucan particles in mice. This study highlights opportunities for further research because only CypA, previously identified as a ligand for DCs, has been investigated among its 20 most abundant components (82). In addition to active vaccination, passive immunotherapy using monoclonal antibodies directed against surface antigens, such as Hsp60 and H2B, has shown potential in experimental models by reducing fungal burden and modulating host immune responses, supporting further exploration as an adjunctive strategy for histoplasmosis (83).

To advance these preventive and therapeutic strategies, a deeper understanding of Hc immune evasion mechanisms and survival pathways is required. The identification of Hc yeast immune evasion and survivability factors has improved our understanding of how Hc could thrive within the human host. Therefore, the development of novel antifungal drugs and vaccines is feasible by utilising this knowledge. A study by Almeida et al. identified several proteins associated with metabolic pathways and enzymes involved in β-glucan elongation as candidates for antifungal drugs and vaccines, respectively (84). Although this study is purely in silico and requires further validation through in vitro and in vivo testing, the potential of utilising crucial proteins and enzymes against Hc survivability opens new possibilities for antifungal drug and vaccine development.

H. capsulatum Strain, Relevance Toward Pathogenesis

Sepúlveda et al. conducted a genomic study on 30 Hc isolates from North America, South America, and Africa, which revealed the presence of several cryptic species, as follows: *H. capsulatum sensu stricto* (Panama), *H. mississippiense* (North America), *H. ohiense* (North America), *H. suramericanum* (South America), and *H. capsulatum* var. duboisii (Africa) (85). A subsequent phenotypic study involving 27 strains representing these species showed distinct species-specific differences, including variation in the α-(1,3)-glucan component in *H. ohiense*, proteolytic activity in *H. mississippiense*, as well as differences in yeast cell size and growth characteristics (86). Another study on *H. suramericanum* revealed a complex, genetically distinct population structure across South America, indicating strong geographic isolation and the possibility of new speciation in this region (87). Together, these genomic and phenotypic findings suggest that genetic divergence among Hc is accompanied by biologically meaningful traits that may contribute to differences in pathogenesis. However, these studies were limited by relatively small sample sizes, a predominance of isolates from the Americas, and a lack of direct correlation with clinical outcomes (85-87).

Based on analyses of a large global dataset of 879 isolates from 47 countries, including 400 sequences analysed using ARF–OLE multilocus typing and 274 sequences included in four-gene analyses (ITS, ARF, OLE, and H-anti), Quan et al. reported a genetically distinct Hc lineage involving isolates from India and Indonesia. These findings suggest a regional population structure among Asian strains. However, the available evidence remains insufficient to support formal taxonomic revision (87, 88). Additionally, current genomic data have not yet systematically demonstrated

consistent differences in disease severity or clinical manifestations across major clades.

Unfortunately, representation from other Asian countries, such as Thailand, Taiwan, Japan, and Malaysia, as well as from Africa, remains very limited. Based on clinical reports and histoplasmin skin test results, exposure to Hc is far more widespread than previously recorded (89). Taiwan, historically not considered endemic, has reported 17 cases of histoplasmosis from 1977 to 2023, including four local cases without a travel history, suggesting local transmission (90). Similar situations occur in other countries, such as Bangladesh, Nepal, and parts of Africa. However, limited testing, low diagnostic capacity, and symptom overlap with tuberculosis lead to the disease being undetected or misdiagnosed (5, 91-93).

Although the genetic diversity of Hc is increasingly recognised, no comprehensive study has directly compared the pathogenesis and clinical relevance of each strain. Consequently, the current strain classification is more taxonomic than clinical. Therefore, further extensive and integrative research combining genomic analysis, clinical data, and pathogenicity studies is needed to better understand the medical impact of Hc strain diversity across different regions worldwide.

Conclusion

A complex interaction occurs between Hc and the host immune response. While the innate and adaptive immune systems can eliminate the fungus in most cases, immune evasion mechanisms allow Hc to survive in hostile host environments and cause disease under certain conditions. Recent reports of histoplasmosis outside the classic endemic regions, such as in parts of Asia and Africa, suggest the existence of distinct strains and alternative pathogenic mechanisms. Advances in proteomic and genomic profiling may facilitate the discovery of novel proteins involved in pathogenesis. Further understanding of these interactions could support the development of improved strategies for prevention, diagnosis, and therapy.

What Is Already Known on This Topic:

Hc is a dimorphic fungus that causes histoplasmosis, a disease ranging from asymptomatic infection to severe disseminated forms. Hc has developed several strategies to evade host immune defences, including modulation of phagolysosomal function, resistance to oxidative stress, and interference with antigen presentation. The host immune response, particularly through innate and adaptive mechanisms, plays a critical role in limiting fungal proliferation. Most data on Hc pathogenesis originate from strains circulating in the Americas, where histoplasmosis is classically endemic. However, recent reports have identified genetically distinct lineages.

What This Study Adds:

This review highlights the complex interactions between the host immune system and H. capsulatum, with particular attention to fungal survival and adaptation under diverse environmental and immunological conditions. The recognition of distinct phylogenetic clades in Asia and Africa suggests potential differences in evolutionary adaptation and pathogenic mechanisms. This review also integrates current findings on cell wall components, host immune responses, and immune evasion strategies of H. capsulatum with their clinical relevance and discusses emerging areas of research with implications for diagnosis and treatment.

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Mini Review
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Genetics of IgA Vasculitis: What We Know and Where We Are Going

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Abstract
Immunoglobulin A (IgA) vasculitis (IgAV) is the most prevalent systemic vasculitis in children. Although the condition is typically self-limiting with spontaneous recovery within a few weeks, both acute and long-term complications can arise, with renal involvement being the most significant. In recent years, considerable attention has been directed toward unraveling the genetic basis of IgAV. Studies have identified associations between disease susceptibility and specific human leukocyte antigen (HLA) polymorphisms. In addition, variants in genes encoding cytokines, chemokines, and other biologically important proteins – particularly those involved in the abnormal glycosylation of IgA1 – have been linked to both increased risk of developing IgAV and more severe disease manifestations. Notably, polymorphisms in the interleukin-1 receptor antagonist (*IL1RN*) and *IL8* genes have been correlated with an increased risk of glomerular injury. Other gene polymorphisms have also been associated with specific clinical phenotypes, such as *HMGB1* and *RAGE*, whereas polymorphisms in genes involved in mucosal immune defense have not demonstrated any significant correlations to date. Ongoing research is essential to clarify these findings further and determine their implications for clinical practice.

Key Words: IgA Vasculitis ▪ Genetics ▪ Human Leukocyte Antigen.

Introduction

Immunoglobulin A (IgA) vasculitis (IgAV), formerly known as Henoch-Schönlein purpura, is the most common systemic vasculitis in children. It typically presents with non-thrombocytopenic palpable purpura, primarily involving the lower extremities and gluteal region, often accompanied by arthritis, nephritis, and/or gastrointestinal symptoms. It is a non-granulomatous systemic vasculitis characterized histologically by leukocytoclastic infiltration of small vessel walls (arterioles, capillaries, and venules) by neutrophils, along with the deposition of immune complexes containing predominantly IgA in the vessel endothelium. These deposits are commonly found in the skin, synovial membrane, and gastrointestinal and urinary tracts (1).

Although IgAV is usually self-limiting and resolves within approximately four weeks, acute complications such as intussusception, gastrointestinal hemorrhage, and bowel perforation may occur. Renal involvement is the most significant long-term complication and primary determinant of morbidity and mortality. Therefore, clinical guidelines recommend follow-up of all IgAV patients for at least 6 to 12 months, even when initial urinalysis and blood pressure are within normal limits (2).

This paper highlights the most significant recent advances in understanding the genetic basis of IgAV and explores their implications for its diagnosis, treatment, and prognosis.

Genetics of IgAV

The genetic basis of IgAV remains incompletely understood, although recent research is increasingly

revealing its underlying mechanisms. Findings from the first genome-wide association studies (GWAS) conducted in IgAV patients of European ancestry suggest that IgAV is strongly associated with polymorphisms in the human leukocyte antigen (HLA) class II region. Notably, significant associations were identified in the intergenic region between *HLA-DQA1* and *HLA-DQB1*, as well as the *HLA-DRB1 11* and *HLA-DRB1 13* loci. The haplotype *DQA101:01/DQB105:01/DRB101:01* was found to confer increased susceptibility to IgAV, with no apparent overlap with the genetic profiles of other autoimmune or autoinflammatory diseases (3, 4).

In addition to HLA-related genes, several non-HLA loci have been implicated in IgAV susceptibility, including genes encoding cytokines (*IL1RN2*, *IL18*, and *TGFB1*), chemokines (*MCP1*), and other functionally relevant proteins (*C1GALT1*, *NOS2A*, *eNOS*, *PON1*, and *MEFV*) (5). Genes involved in the aberrant glycosylation of IgA1, modulation of vascular homeostasis and neoangiogenesis, T-cell function, proinflammatory cytokine activity, and homocysteine metabolism may influence both susceptibility to IgAV and the severity of its clinical course. A shared pathogenic feature of both IgAV and IgA nephropathy (IgAN) is the aberrant O-glycosylation of IgA1, particularly the overproduction of galactose-deficient IgA1 (Gd-IgA1), which promotes immune complex formation and tissue deposition. Polymorphisms in the *C1GALT1* gene may contribute to increased Gd-IgA1 levels, while variants in *C1GALT1C1* can indirectly affect glycosylation. Additionally, genes such as *ST6GALNAC2* and members of the *GALNT* family play critical roles in determining the structure of O-glycans in the IgA1 hinge region (5).

Genetic variants in the interleukin-1 receptor antagonist (*IL1RN*) and *IL8* genes have been associated with an increased risk of renal involvement and glomerular damage in IgAV (6, 7). Conversely, studies examining polymorphisms in the *IL6* gene and genes encoding protein tyrosine phosphatases have not demonstrated significant associations with either disease susceptibility or the risk of renal complications (8, 9).

Sestan et al. conducted whole exome sequencing (WES) in a cohort of patients with IgAV and did not identify any pathogenic variants definitively associated with disease pathogenesis. However, two rare variants of uncertain significance (VUS) were identified: one in exon 3 of the *BAD* gene (c.462G>C, p.Trp154Cys) and the other in exon 5 of the *DHX58* gene (c.560A>G, p.Gln187Arg). These genes are involved in key immune processes – specifically, apoptosis regulation and type I interferon (IFN-I) signaling, respectively. Although the clinical significance of these variants remains unclear, the authors suggest they may warrant further investigation due to their potential role in autoimmune dysregulation (10).

Polymorphisms in genes involved in mucosal immune defense, including *ITGAM–ITGAX* (rs11150612, rs11574637), *VAV3* (rs17019602), *CARD9* (rs4077515), *DEFA* (rs2738048, rs10086 568), and *HORMAD2* (rs2412971), have been implicated in the regulation of IgA production and have been previously identified as risk loci in IgAN. Given their potential relevance, these variants were investigated for their possible roles in IgAV pathogenesis. However, a study involving both adult and pediatric IgAV cohorts found no statistically significant differences in genotype or allele frequencies for these seven polymorphisms, indicating that they may not play a significant role in IgAV susceptibility (11). In a large pediatric cohort of patients with IgAV, at least one *MEFV* gene alteration was detected in 36.5% of cases, with p.E148Q and p.M694V being the most frequently observed variants. *MEFV* variants, known to cause the autoinflammatory disorder familial Mediterranean fever (FMF), may be associated with increased susceptibility to IgAV. Moreover, these variants appear to influence the clinical course of IgAV, as evidenced by their association with hematuria and disease recurrence (12). This potential link is further supported by a case report of an adult patient heterozygous for p.M694I and p.E148Q who manifested a moderate-to-severe form of IgAV (13).

Batnožić Varga et al. performed genotyping using real-time polymerase chain reaction (RT-PCR) and identified several *HMGB1*

polymorphisms associated with specific clinical phenotypes. Homozygous carriers of the rs1412125 polymorphism had a 3.45-fold increased risk of developing IgA vasculitis nephritis (IgAVN). This polymorphism was also linked to multisystem involvement in IgAV, with patients exhibiting purpura, arthritis, nephritis, and gastrointestinal symptoms more frequently carrying the homozygous C/C genotype under a recessive genetic model. Other *HMGB1* polymorphisms showed no significant association with IgAVN or multisystem involvement. However, individuals with the recessive genotypes of rs1045411, rs2249825, and rs1412125 were more likely to develop generalized rash. Conversely, the delT allele of rs41369348 appeared to confer protection against widespread rash. Carriers of either the homozygous T/delT or heterozygous T/delT genotypes of rs41369348 (under dominant and overdominant models) were less likely to develop generalized purpura. Moreover, patients with the heterozygous T/delT genotype were less likely to present initially with palpable purpura. In the same study, the *RAGE* polymorphism rs1800625 was associated with infections preceding IgAV onset, with the heterozygous A/G genotype linked to the highest risk. Additionally, the A/T genotype of rs1800624 was associated with a lower likelihood of initial skin manifestations (14).

Conclusion

IgAV is a relatively common disease in children compared to adults. While its genetic architecture remains only partially defined, pathogenesis appears to involve both HLA class II region variants and non-HLA genes related to immune and vascular regulation, suggesting a complex genetic predisposition (3-5). Genetic polymorphisms in *IL-1* and *IL-8* may influence renal involvement, while variants in *IL-6* and protein tyrosine phosphatase genes appear unrelated to disease susceptibility or severity (8, 9). WES in patients with IgAV revealed no definitive pathogenic variants, although rare variants in the *BAD* and *DHX58* genes warrant further study for their potential role in immune dysregulation (10). Moreover, mucosal immune defense polymorphisms do not represent novel genetic risk factors for IgAV pathogenesis (11). Alterations in the *MEFV* gene, along with *HMGB1* and *RAGE* polymorphisms, also play a potential role in the development and clinical course of IgAV (12-14). Further research is needed to clarify these associations and their clinical relevance.

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Case Report and Review of Literature
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Glycogen-Rich Clear Cell Carcinoma of the Breast: Report of Two New Cases and an Updated Literature Review*

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Abstract

Objective. To report two additional cases of glycogen-rich clear cell carcinoma (GRCC) of the breast – detailing their clinicopathologic features, immunophenotypes, and follow-up – and to provide an updated literature review since 2020. **Case Reports.** Two patients (66 and 52 years old) had GRCC confirmed morphologically and histochemically. Case 1 was ER-positive/HER2-positive (luminal B/HER2-positive) and was managed with surgery, followed by adjuvant chemotherapy, endocrine therapy, and anti-HER2 therapy (trastuzumab). Case 2 was triple-negative and received neoadjuvant chemoimmunotherapy (pembrolizumab-based) with marked pathologic tumor regression at resection. Both patients were disease-free at one and 12 months, respectively. **Conclusions.** GRCC is heterogeneous and should not be regarded as a single clinicopathologic entity within invasive breast carcinoma of no special type or assumed to have a uniform prognosis. Management should be biomarker-guided, as illustrated by these cases. The role of targeted and immune therapies in GRCC warrants multi-institutional studies.

Key Words: Breast Cancer ▪ Special Patterns ▪ Glycogen-Rich Pattern ▪ Biomarkers ▪ Outcome.

Introduction

Invasive breast carcinoma is the most common malignancy in women worldwide (1). It is a biologically and morphologically heterogeneous disease that comprises more than 20 histologic subtypes, of which invasive breast carcinoma of no special type (IBC-NST) is the most frequent (≈70–80%) (2). Special types account for approximately 10–20% and include lobular, tubular, mucinous, medullary, and several rarer variants, each with distinctive morphologic and molecular features that may influence clinical management and prognosis (2, 3).

Within this spectrum, a subset of rare tumors exhibits clear-cell cytomorphology—so-called breast carcinomas with clear-cell features or patterns. Importantly, this appearance is etiologically diverse: cytoplasmic clearing may indicate different intracellular contents or even a processing artifact (2, 3). Determining the nature of the optically clear cytoplasm is critical for at least two reasons: (1) clear change can be artifactual, and (2) when clearing involves more than a few cells, understanding its biochemical basis helps guide classification. Upon confirmation, invasive carcinomas with clear cytoplasm can be further categorized as lipid-rich carcinoma, secretory carcinoma, apocrine carcinoma with clear (histiocytoid) cytoplasm,

*The preliminary data from this study were presented at the 37th European Congress of Pathology (ECP), which was held in Vienna (Austria) between September 6 and 10, 2025.

and glycogen-rich clear-cell pattern of IBC-NST (2, 4-7).

Glycogen-rich clear cell carcinoma (GRCC), first described by Hull et al. in 1981 (8), is characterized by tumor cells with abundant intracytoplasmic glycogen that is Periodic Acid-Schiff (PAS)-positive and PAS-diastase-sensitive (i.e., PAS staining is abolished after diastase digestion) (2, 4). Because clear cytoplasm alone does not prove the presence of glycogen and glycogen is not the sole cause of clearing, both morphology and histochemistry are required. Evolving diagnostic criteria stipulate demonstration of PAS positivity and PAS-D sensitivity in ≥90% of tumor cells to assign a glycogen-rich pattern (2).

Although early reports labeled GRCC highly aggressive, accumulating series suggest a variable clinical course (9). A population-based SEER analysis (155 GRCC vs >1.2 million non-GRCC breast cancers) found that GRCCs are more often high-grade, present at advanced stage, more frequently triple-negative, and are associated with worse survival than non-GRCC cancers (9), underscoring their distinct prognostic profile (2, 10). GRCC/clear-cell pattern of IBC-NST is rare, with reported incidences ranging from 0.01% to 3% (9, 11-13). Owing to its rarity and a literature dominated by case reports and small series, GRCC remains understudied relative to other subtypes.

Here, we report two new GRCC cases of the breast, detailing their clinicopathologic features, immunoprofiles, and follow-up. We also provide an updated literature review (PubMed/MEDLINE, Google Scholar, Web of Science, and Scopus) of GRCC that have been published since our previous review in 2020 until October 2025 (Table 1) (4).

Case Presentations

We report two invasive breast carcinomas with clear-cell morphology on hematoxylin–eosin sections. Intracytoplasmic glycogen was confirmed by periodic acid–Schiff (PAS) positivity that was diastase-labile on PAS-D. Immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki-67 was performed using standard protocols (14, 15). The tumor-infiltrating lymphocytes (TIL) were assessed following

Table 1. Review of the Clinicopathologic Characteristics of Glycogen-rich Clear Cell Carcinomas Published in the Previous Five Years (2020-2025)

Author (year)	No of cases	Age (yrs)	Diagnosis	AJCC TNM	Grade	IHC profile	Ki-67	PAS/PAS-D
Liu et al. (2020)	6	46-57	GRCC	n/a	n/a	ER-, PR-, HER2 0 (3/6)	n/a (%)	Positive/ sensitive
Georgescu et al. (2021)	2	64 54	GRCC	pT1cN2a pT1cN0	G3 G2	ER+, PR+, HER2 0 ER+, PR+, HER2 1+	25 5	Positive/ sensitive
Demyashkin et al. (2021)	9	n/a	GRCC	pT1 (1/9) pT2 (6/9) pT3 (2/9)	G3	ER and PR negative (9/9) ~80% HER2 3+	26*	Positive/ sensitive
De la Sancha et al. (2021)	1	69	GRCC	pT1cN0	G2	ER+, PR-, HER2 0	n/a	Positive/ sensitive
Sanjeeviah et al. (2022)	1	41	GRCC	pT2N3a	G2	ER-, PR-, HER2 3+	50	Positive/ sensitive
Singh et al. (2022)	1	70	GRCC	pT1N0	G2	ER+, PR+, HER2 0	20	Positive/ sensitive
Lee et al. (2022)	1	79	GRCC	pT2N0	n/a	ER+, PR-, HER2 0	n/a	Positive/ Sensitive
Braganza et al. (2025)	1	75	GRCC	pT2	G3	ER+, PR-, HER2 2+ (not amplified)	25	Positive/ sensitive

GRCC = Glycogen-rich clear cell carcinoma; TNM = Tumor node metastasis; IHC = Immunohistochemical; PAS = Periodic acid–Schiff; PAS-D = PAS diastase; ER = Estrogen receptor; PR = Progesterone receptor; HER2 = Human epidermal growth factor receptor 2; n/a = Not available; *The study reported the average Ki-67 values.

the recommendations of the International TILs Working Group 2014 (16). Tumors were classified as GRCC when ≥90% of tumor cells exhibited clear cell morphology and contained intracytoplasmic glycogen, confirmed by special stains (PAS and PAS-D) (2). None of the cases were sequenced by next-generation sequencing (NGS) for targeted treatment purposes.

Case 1 (GRCC, Luminal B, HER2+)

A 66-year-old woman with a positive family history detected a left breast mass on self-examination. Ultrasound demonstrated a suspicious anechoic focus in the upper outer quadrant (~10 mm), and mammography confirmed a poorly circumscribed mass with microcalcifications. Because of a documented allergy to local anesthetics, a core biopsy was not performed; at the patient's request, a total mastectomy with axillary dissection was undertaken. Grossly, a 13 mm tumor was identified in the upper outer quadrant near the deep margin. Microscopically, the carcinoma was predominantly solid, with clear-cell morphology in >90% of cells (Figure 1A-C). Immunohistochemistry showed that the tumor cells were diffusely (100%) and strongly positive for ER (Figure 1D), negative for PR (0%), while HER2 protein exhibited a complete, intense membranous expression in >90% of cancer cells (IHC score 3+) (Figure 1E); the proliferating marker Ki-67 was positive in ~10% of cancer cells. Tumor-infiltrating lymphocytes were low (~1%) (Figure 1A). The tumor cells were strongly and diffusely PAS-positive and PAS-D–sensitive, consistent with intracytoplasmic glycogen (Figure 1F). An associated solid clear-cell ductal carcinoma in situ (DCIS) comprised ~10% of the lesion (Figure 1A arrow). All axillary nodes were negative (AJCC stage pT1cN0Mx). Adjuvant therapy included 12 weekly cycles of paclitaxel combined with trastuzumab (administered every three weeks) for one year, followed by radiotherapy. Endocrine therapy with letrozole was initiated after the completion of both adjuvant chemotherapy and radiotherapy. The patient remains disease-free at 1-year follow-up.

Case 2 (GRCC, Triple-Negative)

A 52-year-old woman noted a left periareolar mass after a fall. Initial ultrasonography suggested a fibroadenoma, and short-interval follow-up (four weeks) was advised. During this period, the overlying skin became inflamed, prompting a second opinion. Mammography then demonstrated a circumscribed, lobulated mass measuring 31 × 28 mm. Core needle biopsy confirmed a high-grade, invasive breast carcinoma. Carcinoma was predominantly solid, with clear cell morphology in more than 90% of cells (Figure 2A-C), with a triple-negative immunophenotype (ER-/PR-/HER2 1+) (Figure D, E), and a high Ki-67 (90%). The cells were diffusely PAS-positive and PAS-D-sensitive, consistent with intracytoplasmic glycogen accumulation (Figure 2F). TIL levels were low (~5%, Figure 2B). PD-L1 testing was not performed. The multidisciplinary tumor board recommended neoadjuvant chemo-immunotherapy. The patient received paclitaxel and carboplatin for 12 weeks, followed by four cycles of the AC protocol (doxorubicin, cyclophosphamide). This was combined with the PD-1 inhibitor pembrolizumab, administered every three weeks. Of note, the patient obtained pembrolizumab through self-funding, as access to this therapy was restricted and subject to prolonged waiting periods under the Federal Solidarity Fund program. Subsequent clinical and ultrasound assessments showed marked tumor regression. The patient underwent segmentectomy with axillary dissection. Grossly, a 5-mm residual lesion was present between the lateral quadrants within a 31 × 28 mm tumor bed. The entire tumor bed was submitted for microscopic assessment. Postoperative pathology was evaluated using the MD Anderson Residual Cancer Burden (RCB) method (17, 18). The tumor response to neoadjuvant therapy was classified as RCB-II, indicating a partial pathologic response. The tumor bed showed treatment-related changes (inflammatory infiltrates, hemorrhage, fat necrosis). Residual invasive carcinoma was confined to a single block and displayed clear-cell morphology with therapy-related atypia (marked pleomorphism, hyperchromasia)

(Figure 3A-B). Ductal carcinoma in situ was not identified. All axillary lymph nodes were negative (AJCC ypT1aN0Mx). The patient is scheduled for adjuvant radio- and systemic therapy, as recommended by the multidisciplinary tumor board (as of early October 2025).

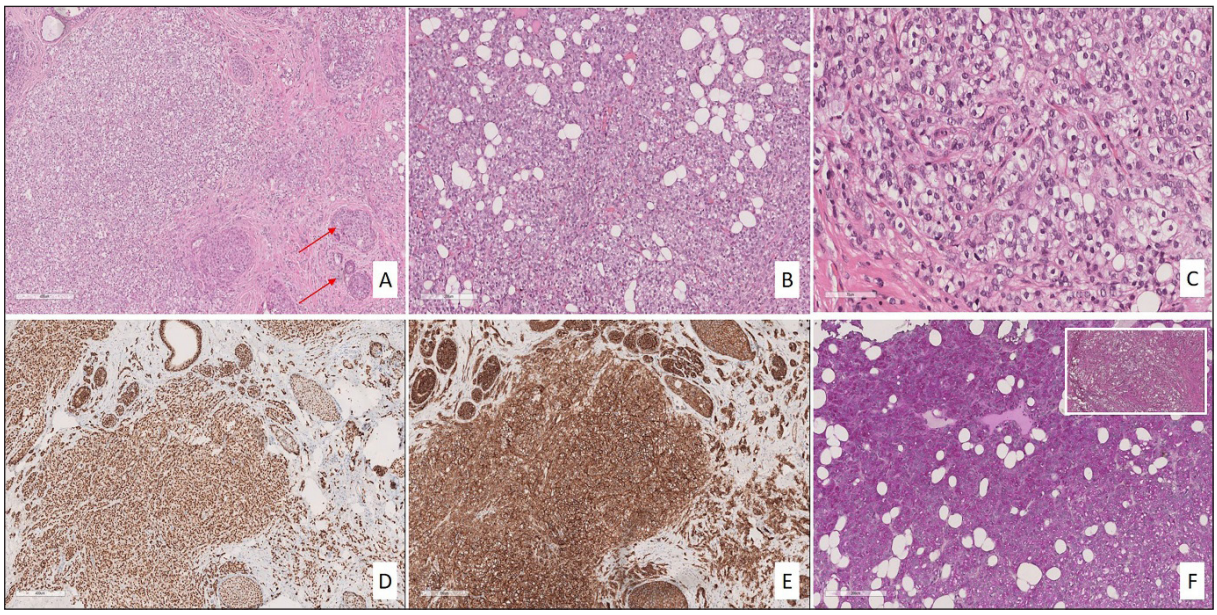

Figure 1A–F. Case 1. Clear-cell morphology in >90% of tumor cells on hematoxylin and eosin-stained slides (A, 4×; B, 10×; C, 20×). Tumor cells are diffusely and strongly ER-positive (D) and HER2-positive (E). Clear cells are strongly PAS-positive (F) and PAS-D–sensitive (inset in F), consistent with intracytoplasmic glycogen.

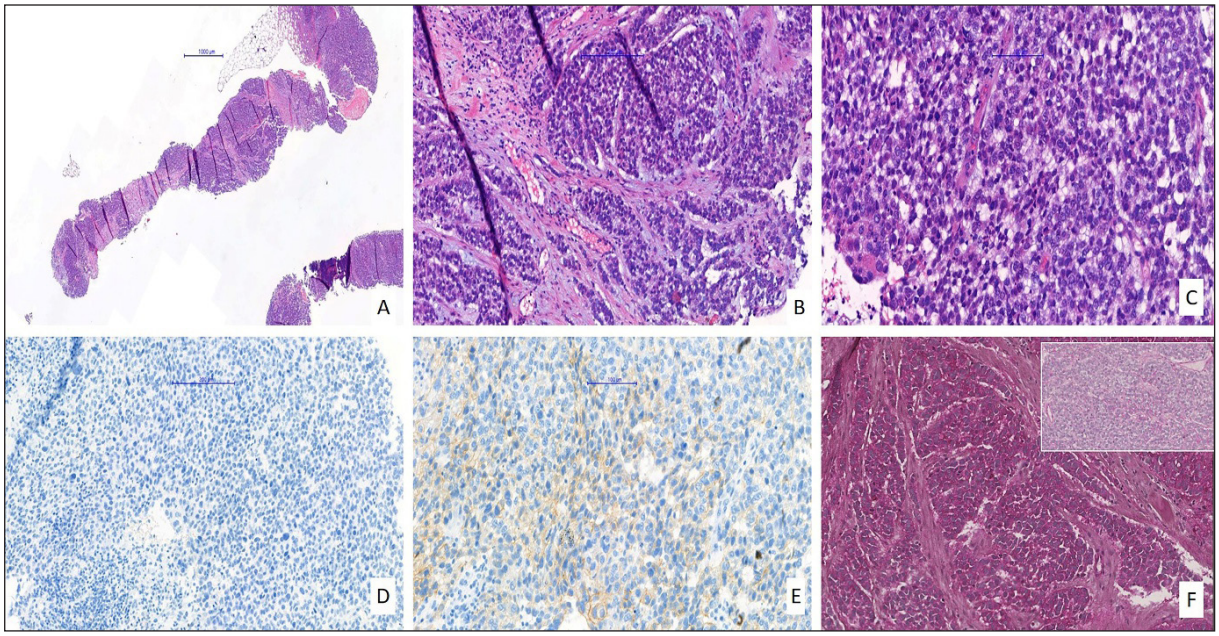

Figure 2A–F. Case 2. Core biopsy showing invasive carcinoma with predominant clear-cell morphology on hematoxylin and eosin-stained slides (A, 4×; B, 10×; C, 20×). Tumor cells are ER-negative (D) and HER2 negative/low (score 1+) (E). Clear cells are strongly PAS-positive (F) and PAS-D–sensitive (inset in F), consistent with intracytoplasmic glycogen.

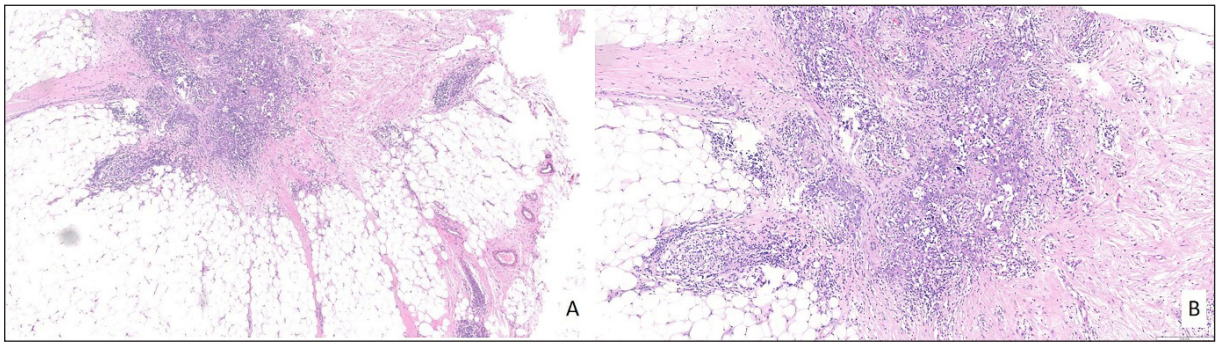

Figure 3A–B. Case 2, post-neoadjuvant therapy. Residual clear cell carcinoma with prominent inflammatory response and fibrosis on hematoxylin and eosin-stained slides (A, 4×; B, 20×).

Discussion

The two cases presented here illustrate the morphologic unity but biological diversity of GRCC of the breast—a rare histologic variant (pattern) of invasive breast carcinoma characterized by clear cytoplasm due to intracytoplasmic glycogen. Despite nearly identical H&E appearances, our patients showed strikingly different immunophenotypes and proliferation indices (Case 1: ER-positive, PR-negative, HER2 3+, low Ki-67; Case 2: triple-negative with 90% positive Ki-67). These observations reinforce that GRCC is not a biologically uniform entity but rather a heterogeneous group with potentially distinct molecular pathways and clinical outcomes (10). To contextualize our experience, we searched for newly diagnosed GRCCs from 2020 to 2025; results are summarized in Table 1 (13, 19-25).

Reported incidence varies widely, from <1% in population-based registries (SEER) to ~0.1–3% in some single-institution series from the 1980s–2020s (7, 9, 12, 13, 26-28). By definition, GRCC shows glycogen in ≥90% of tumor cells, documented by PAS positivity that is abolished after diastase digestion (PAS-D) (2). While most tumors show conventional ductal architecture, described patterns include solid papillary, tubular, mixed morphologies, and occasional neuroendocrine-like features (29). Radiologic findings are nonspecific; some reports note prominent microcalcifications on mammography and a peripheral "halo" on MRI, but no pathognomonic pattern has been established (30, 31).

Several case series report a predominance of luminal tumors (ER/PR positive, HER2 negative); for example, Kuroda et al. found ≈56% luminal A among reviewed cases (28). Our series illustrates the spectrum, with one luminal B/HER2-positive (ER+/PR–/HER2+) carcinoma and one triple-negative tumor. Across studies, HER2 positivity varies widely—from rare/low (3/28 in Ma et al.) (32) to as high as ≈44% in Akbulut et al. (33) and ~80% in the study of Demyashkin et al. (13) — likely reflecting differences in cohort selection, testing methodology, and sample size. Systematic, standardized studies are needed to clarify the molecular underpinnings of GRCC and its relationship to IBC-NST.

Our second case showed a triple-negative phenotype with low TILs but a high Ki-67 proliferative index. Given limited morphology-based evidence in the literature, treatment followed standards for triple-negative breast cancer (TNBC) rather than GRCC-specific features. Tumor-cell glycogen can function as an energy buffer that promotes therapeutic resistance and survival (34). This case illustrates that GRCC may occasionally present as high-grade, triple-negative, and TIL-low, suggesting a metabolically driven rather than immunogenic tumor. To our knowledge, this is among the first reported triple-negative GRCCs treated with neoadjuvant chemo-immunotherapy (pembrolizumab). The rationale for PD-1 blockade stems from the known immunogenicity of TNBC and the demonstrated benefit of adding pembrolizumab to multi-agent chemotherapy in the neoadjuvant setting (KEYNOTE-522), which improved response

rates and long-term outcomes versus chemotherapy alone (35). Recognizing this pattern is important, as its biology and therapeutic response may differ from classic basal-like TNBC (2, 28).

Neither of our cases showed lymph-node metastasis. Early reports (7, 8) and a population-based analysis by Zhou et al. (9) associated GRCC with higher grade, more advanced stage, a triple-negative shift, and poorer survival compared with non-GRCC breast cancers (7, 9). In contrast, Georgescu et al. found no significant prognostic differences between GRCC and non-GRCC cohorts (20). Individual long-term observations by Sanjeeviah et al. (19) further suggest that outcomes may be independent of clear-cell morphology per se and can be unexpectedly favorable. The follow-up in both cases presented in our study was short (1-12 months); therefore, long-term outcomes and prognostic assessments cannot be reported.

One of the important issues highlighted in our study is limited access to targeted drugs in the Federation of Bosnia and Herzegovina. Our patient (case 2, triple-negative carcinoma) had to obtain pembrolizumab through self-funding, given the limited utility and prolonged waiting period for access to immunotherapy in the Federation of Bosnia and Herzegovina. This common problem with a substantial adverse effect on cancer patient treatment and outcome requires urgent public action and has been previously discussed in the literature (36-40).

GRCC is heterogeneous and should not be regarded as a single clinicopathologic entity within IBC-NST or assumed to have a uniform prognosis. Management should be biomarker-guided, as illustrated by these cases. The role of targeted and immune therapies in GRCC warrants multi-institutional studies.

What Is Already Known on This Topic:

Glycogen-rich clear cell carcinoma (GRCC) of the breast is a rare morphologic variant characterized by clear cytoplasm due to intracytoplasmic glycogen, confirmed by PAS positivity that is diastase-sensitive; most series define GRCC when ≥90% of tumor cells show this feature. Reported incidence is very low (<1%), and the literature is dominated by small series and case reports. GRCC shows biologic heterogeneity: many cases are luminal (ER/PR-positive, HER2-negative), but HER2 positivity and triple-negative phenotypes have also been described; population-based data suggest higher grade, more advanced stage, and potentially worse survival than non-GRCC, although these findings are not uniform across studies. No pathognomonic imaging pattern is established, and management is not GRCC-specific – treatment generally follows biomarker-guided breast cancer standards.

What This Study Adds:

We describe two pathologically confirmed glycogen-rich clear cell carcinomas (GRCC) with contrasting biomarker profiles and clinical courses: One ER+/PR–/HER2 3+, low Ki-67 case and one triple-negative, high Ki-67 case treated with pembrolizumab-based neoadjuvant chemoimmunotherapy showing marked regression. These findings reinforce the biologic heterogeneity of GRCC and the need for biomarker-guided management. We document coexistent clear-cell DCIS in one case and low TILs in both cases. We also provide an updated 2020–2025 literature review, underscoring gaps that warrant larger, multi-institutional studies and prospective molecular profiling of this peculiar malignancy.

Authors' Contributions: Conception and design: JR and SV; Acquisition, analysis, and interpretation of data: JR, FS, NI, TC, SB, ZG and SV; Drafting the article: JR and SV; Revising it critically for important intellectual content: JR, FS, NI, SB, TC, ZG and SV; Approved the final version of the manuscript: JR, FS, NI, SB, TC, ZG and SV.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
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Pediatric Spitzoid Melanoma: A Case Report

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Abstract

Objective. To highlight the diagnostic and therapeutic challenges of Spitzoid melanoma in childhood, with a focus on its potential genetic predisposition. **Case Report.** A 7-year-old female patient presented with a growing nodular lesion on her upper leg. Excision was performed, and histopathological analysis confirmed a diagnosis of Spitzoid melanoma, classified as pT2a. Following a multidisciplinary review, wide local re-excision and sentinel lymph node biopsy (SLNB) were recommended. No residual tumor was found, and the SLNB was negative. A comprehensive diagnostic evaluation ruled out systemic disease, and no additional treatment was required. Germline genetic testing identified a pathogenic *CHEK2* variant (c.444+1G>A), prompting recommendations for genetic counseling and close follow-up. **Conclusion.** This case report contributes to the limited body of knowledge on pediatric Spitzoid melanomas and underscores the importance of genetic insights in guiding both diagnostic and treatment decisions. The detection of a *CHEK2* mutation underscores the importance of genetic profiling in family counseling.

Key Words: Melanoma ▪ Spitz Tumor ▪ Child, Genomics.

Introduction

Spitzoid melanoma is a rare pediatric neoplasm that presents a diagnostic challenge owing to its clinical and histopathological similarities with benign Spitz nevi and atypical Spitz tumors (1). While traditional histopathological assessment remains essential for diagnosis, advances in molecular genetics have provided valuable insights into identifying key mutations, such as *BRAF*, *NRAS*, and kinase fusions, which assist in tumor classification and risk stratification (2). Despite these advancements, the role of germline mutations, including Checkpoint kinase 2 (*CHEK2*) mutations, in pediatric Spitzoid melanoma remains unclear (3).

This case reveals a rare pathogenic *CHEK2* variant in a child with Spitzoid melanoma, highlighting the value of genetic profiling in guiding treatment and follow-up for this rare tumor.

Case Report

A 7-year-old female patient was referred to a pediatric hematologist after a 5 mm nodular lesion on her right upper leg was excised at a private clinic. Six months prior, a reddish-gray skin change, approximately 1 mm in size, was observed. A month before the excision, growth and color changes were observed, with the lesion almost tripling in volume and changing color to a brownish hue, accompanied by a central grayish area. The child had no complaints. The family history was negative for atypical moles, melanoma, or other malignancies.

The excised skin biopsy measured 5 × 4 × 1 mm and had a central grayish papule approximately 3 mm in diameter. Histopathological analysis revealed the accumulation of large melanocytes, primarily within the dermis, with some located in the epidermis. The cells exhibited an epithelioid

appearance with a high nucleus-to-cytoplasm ratio, and their cytoplasm was abundant in pigment. Immunohistochemical staining revealed a medium-high Ki-67 proliferation index extending to the base of the lesion, along with a diffuse loss of p16 expression (Figure 1).

The finding was consistent with Spitzoid melanoma, exhibiting a Breslow depth of 1.77 mm and Clark level III. The tumor exhibited vertical growth and mitotic activity, with no signs of lymphatic invasion. Based on the pathological findings, it was classified as pT2a. The case was reviewed by a multidisciplinary team, which recommended re-excision of the lesion with wide margins, sentinel lymph node biopsy (SLNB), and genetic testing for cancer predisposition genes.

Re-excision was performed, and the specimen measured 4 × 2.8 ×1.2 cm. Serial sections showed no residual tumor. Hematoxylin and eosin (HE) staining and immunohistochemistry with S-100 and HMB-45 showed no tumor infiltration in the sentinel lymph node. Pathological review of the primary excision at another institution confirmed the diagnosis of Spitzoid melanoma. To assess potential metastases, comprehensive imaging studies, including abdominal, pelvic, and inguinal ultrasound, chest X-ray, chest computed tomography, and lymphoscintigraphy, were performed. All imaging studies showed no evidence of metastatic disease. As the malignancy was limited to the excised lesion, no additional treatment was indicated beyond the surgical excision. Genetic testing for hereditary cancer syndromes using next-generation sequencing (NGS) identified a heterozygous pathogenic variant in the *CHEK2* gene (c.444+1G>A). Variants of uncertain significance (VUS) were detected in the *PMS2*, *FLCN*, and *FANCI* genes. Genetic counseling was recommended. The father was unavailable for testing, and the mother tested negative for *CHEK2* mutations. The patient was followed up regularly and remained in remission three years after diagnosis.

Discussion

Spitz tumors, which encompass a wide range of melanocytic lesions from benign nevi to melanoma, have a specific histomorphology and distinct

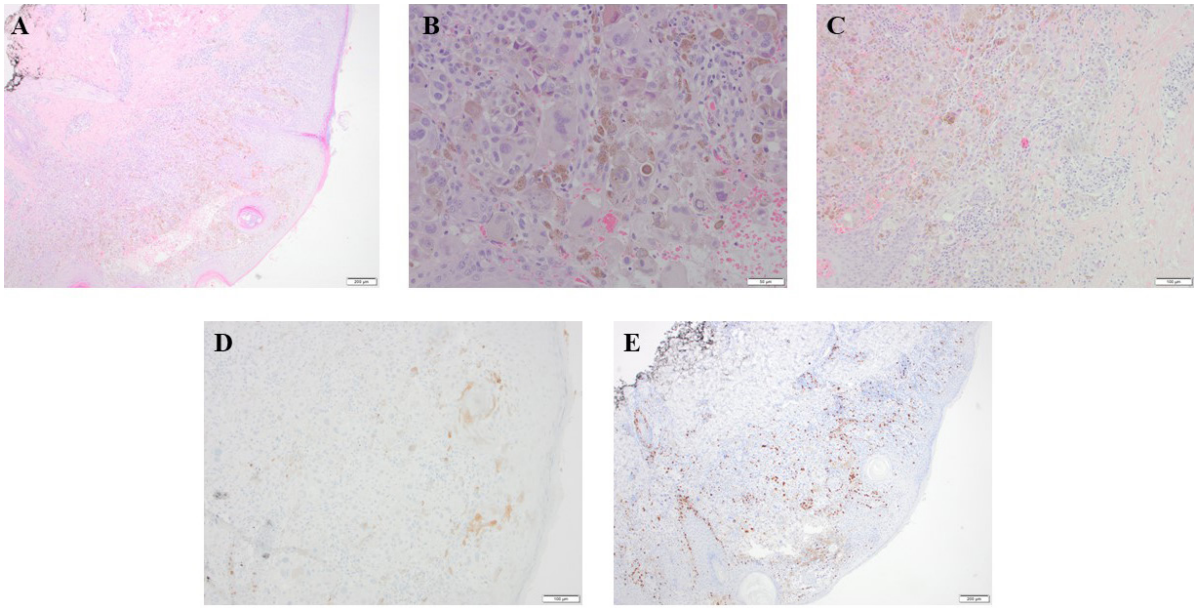

Figure 1. Spitzoid melanoma. A. Predominantly dermal tumor composed of coalescing nests and sheets of large epithelioid melanocytes with pleomorphic nuclei and prominent nucleoli (H&E, 40×). B. Large epithelioid melanocytes interspersed with lymphocytes (H&E, 200×). C. Deep dermal component lacking melanocytic maturation, with tumor cells surrounded by lymphocytic infiltrate (H&E, 100×). D. Loss of immunohistochemical p16 staining (IHC, p16, 100×). E. Moderate-to-high Ki-67 expression in tumor cells (IHC, Ki-67, 100×).

molecular pathways. Despite these features, standardized classification criteria for Spitz lesions are often lacking, leading to diagnostic uncertainty among pathologists and considerable variability in treatment recommendations (4). The 2023 World Health Organization classification of skin tumors sub-classifies Spitz tumors into three groups: Spitz nevi, Spitz melanocytoma (previously defined as atypical Spitz tumor), and Spitz melanoma (5). Unlike conventional nevi and melanomas, most Spitz melanomas are not clinically distinguishable from Spitz nevi. Spitz nevus typically presents in childhood or adolescence as a well-circumscribed, dome-shaped, pink to red papule or plaque, most commonly located on the face or lower extremities. It is composed of large epithelioid and/or spindle-shaped melanocytes (4).

There are no universally accepted criteria for distinguishing Spitz nevi from Spitz melanocytoma; however, several features have been proposed, including lesion size, degree of nuclear pleomorphism, depth, and predominance of large cell aggregates at the base of the lesion (6). Spitz melanomas often present as rapidly growing or color-changing nodules or polypoid lesions. They are commonly larger than Spitz nevi or Spitz melanocytoma, with diameters of 10 mm or more. While they can occur at any anatomical site, pediatric cases frequently present on the limbs. They are frequently amelanotic or exhibit ulcerated bleeding surfaces that mimic pyogenic granulomas. Notably, the ABCD criteria (asymmetry, border, color, and diameter) employed for assessing conventional melanoma are observed in fewer than 50% of Spitz melanomas, limiting their diagnostic reliability (7).

Merkel and colleagues introduced the term "Spitzoid melanoma of childhood" to distinguish them as a group of melanomas with a favorable prognosis. Based on their experience, only a small proportion of affected children develop lymph node or in-transit metastases, whereas distant metastases are exceptionally rare (8). Raghavan et al. proposed that the terms "Spitzoid melanoma" and "Spitz melanoma", although often used interchangeably, should be regarded as referring to distinct entities. Spitzoid melanoma generally refers to melanomas that exhibit characteristic morphology, comprising large epithelioid or spindle-shaped cells with abundant eosinophilic cytoplasm. In contrast to this purely morphology-based diagnosis, Spitz melanoma specifically refers to melanomas harboring genetic alterations characteristic of the Spitz lineage (2). Recent advancements in molecular biology have revolutionized our understanding of melanomagenesis. Up to 80% of Spitz neoplasms harbor oncogenic fusions involving either receptor tyrosine kinase genes (such as *ALK, FGFR1, MET, MERTK, NTRK1/2/3, RET,* and *ROS1*) or serine-threonine kinase genes (such as *BRAF, ERBB4, MAP3K3, MAP3K8,* and *PRKDC*). Importantly, these kinase fusions are mutually exclusive, and the fusion transcript is highly expressed in most cases (5). Therefore, an integrated approach combining morphological evaluation and genomic analysis is likely to remain the diagnostic standard for Spitz lesions.

Our patient presented with a reddish-gray, minimally pigmented limb lesion that was excised due to its growth and color change; however, the excision was inadequate. Immunohistopathology confirmed the diagnosis of Spitzoid melanoma. Given its rarity and the lack of advanced molecular techniques, a second opinion was sought, which confirmed the diagnosis. Wide re-excision and SLNB were performed. Although SLNB has been proposed for the management of these lesions, its role and prognostic value remain the subject of debate in pediatric practice (8). Cerrato et al. reported a 100% survival rate in pediatric SLNB-positive and regional lymph node-positive Spitzoid melanoma and melanocytoma (9). Batra proposed that most primary lesions can be managed with excision or re-excision with negative margins, followed by clinical follow-up and serial ultrasound monitoring of regional nodes (10). Since the lesion in our patient was localized and completely excised with clear margins, adjuvant therapy was not indicated.

In recent years, multiple germline pathogenic variants associated with an increased risk of skin malignancies have been identified, several of which are implicated in hereditary tumor predisposition syndromes. Spitz morphology in familial

melanoma has been associated with germline variants in the *POT1*, *TERF2IP*, *ACD*, and *TERT* genes (11).

Genetic testing of our patient revealed a heterozygous pathogenic variant in the *CHEK2* gene (c.444+1G>A). *CHEK2* is a tumor suppressor gene that encodes the protein CHK2, a serine-threonine kinase, involved in the cellular DNA damage response. The association of germline *CHEK2* variants has been confirmed in breast, prostate, kidney, thyroid, and colon cancers (12). Several studies have reported an increased risk of melanoma in individuals with *CHEK2* mutations (13-15). Although their exact role remains unclear, they may contribute to the risk of Spitz melanoma due to their involvement in cell cycle regulation and tumorigenesis (15). The identification of the pathogenic *CHEK2* variant in our patient prompted counseling and facilitated a comprehensive follow-up.

This case underscores several important considerations. It is essential for clinicians to maintain a high level of suspicion and include melanoma in the differential diagnosis of atypical or evolving skin lesions in children. Moreover, amelanotic or hypopigmented melanomas can mimic a range of other conditions, causing diagnostic delays or inappropriate biopsy or excision techniques. While Spitzoid melanoma can occur at any anatomical site, pediatric cases frequently present on the limbs, necessitating vigilance when examining lesions in these areas (7). The diagnosis of Spitzoid melanoma presents significant challenges in terms of histopathology. If molecular techniques for analyzing tumor tissues are not available, a review at specialized centers is highly recommended. In the genomic era, germline testing, which identifies novel pathogenic variants, can serve as a valuable tool for managing and monitoring affected children.

Conclusion

This case underscores the diagnostic and therapeutic challenges associated with childhood Spitzoid lesions. The identification of a pathogenic *CHEK2* variant emphasizes the value of germline genetic testing in atypical cases and raises important questions about its role in melanocyte biology. Although SLNB and wide local excision remain the standard approaches, our case supports the need for individualized management and follow-up strategies.

What Is Already Known on This Topic:

Spitzoid melanoma is an extremely rare childhood malignancy that presents diagnostic challenges owing to its shared clinical and histopathological features with benign Spitz nevi and Spitz melanocytoma. Amelanotic melanoma and its hypopigmented variants further complicate clinical diagnosis. Clinicians screening for melanoma should maintain a high level of suspicion and include it in the differential diagnosis of red skin lesions. Recent advances in molecular techniques have significantly improved the diagnosis, classification, and understanding of the underlying mechanisms of skin tumors in children.

What This Study Adds:

This study highlights the importance of tumor genetic profiling in improving diagnostic accuracy. Germline genetic testing plays a crucial role in identifying factors that may be implicated in melanoma predisposition and assessing long-term cancer risk. The identification of a pathogenic CHEK2 variant contributes to the growing body of evidence supporting molecular analysis as a crucial tool for managing complex melanocytic lesions in children. Close interdisciplinary collaboration between pediatric oncologists, pathologists, dermatologists, and geneticists is essential for managing these patients.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
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Early Detection of Inferolateral Ischemia Using a Smartphone-Based ECG Device: A Case of Triple-Vessel Disease Confirmed by Coronary Angiography

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Abstract

Objective. This case report describes the capability of a smartphone-based electrocardiogram (ECG) in detecting multivessel coronary artery disease (CAD), with initial findings suggestive of double-vessel involvement, which was later confirmed as triple-vessel disease (TVD) by coronary angiography. **Case Report.** In this case report, we describe a 51-year-old woman with a known medical history of CAD, hypertension, TVD, and a prior episode of acute coronary syndrome who presented to Swami Rama Himalayan University, Dehradun, with complaints of chest pain. She had previously undergone percutaneous coronary intervention with stent placement. Conventional 12-lead ECG (Philips PageWriter ECG) indicated myocardial ischemia. Follow-up smartphone-based ECG (Spandan Pro) revealed inferolateral ischemia possibly affecting the left anterior descending artery (LAD) and left circumflex artery (LCX), with a possible diagnosis of double-vessel disease (DVD). Coronary angiography later confirmed the diagnosis of TVD with significant stenosis of the LAD, LCX, and right coronary artery, along with additional involvement of the left main coronary artery. Post-angiography, the patient was recommended for coronary artery bypass grafting as the first option and percutaneous transluminal coronary angioplasty as an alternative. **Conclusion.** This case illustrates the clinical efficacy of the smartphone-based ECG device in detecting inferolateral ischemia suggestive of DVD in patients with suspected or known CAD and highlights its diagnostic concordance with standard investigations, particularly coronary angiography.

Key Words: Inferolateral Ischemia ▪ Portable ECG ▪ Coronary Artery Disease ▪ Vessel Disease ▪ Coronary Angiography.

Introduction

Coronary artery disease (CAD) continues to be the leading cause of death worldwide (1). The severe form of CAD, known as triple-vessel disease (TVD), affects blood flow to the left anterior descending (LAD), left circumflex (LCX), and right coronary artery (RCA). An estimated 30% of individuals with CAD are thought to have TVD, which has a significant mortality risk and necessitates prompt risk stratification (2). The observation of ST deviations in patient electrocardiograms (ECGs) is a critical technique for diagnosing myocardial ischemia (3). The 12-lead ECG is a highly accepted diagnostic tool for diagnosing ischemia. Nevertheless, conventional 12-lead ECGs are typically utilized in hospital settings, thus limiting their application to regular monitoring and early detection in remote or developing regions (4). This highlights the need for a portable, readily available ECG device that can effectively augment diagnostic capabilities beyond conventional healthcare centers.

One such device is the Spandan Pro, a portable smartphone-based 12-lead ECG device developed by Sunfox Technologies Private Limited (Dehradun, India). Spandan Pro ECG is a medical

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device that has been certified and complies with important international and Indian regulatory standards. It holds certification under the Indian Medical Device Rules (MDR) as regulated by the Central Drugs Standard Control Organization (CDSCO) and maintains a certified Quality Management System (QMS) compliant with ISO 13485. Furthermore, the device adheres to critical international safety and performance standards: IEC 60601-1 (General requirements for basic safety and essential performance), IEC 60601-1-2 (Electromagnetic compatibility - EMC), and IEC 60601-2-25 (Particular requirements for the basic safety and essential performance of electrocardiographs). The device has a Computerized Algorithm for the detection of ST-Elevation Myocardial Infarction (STEMI) or Non-ST-Elevation Myocardial Infarction (NSTEMI), and Arrhythmia, and it is formally validated in accordance with the international standard for diagnostic electrocardiographs, IEC 60601-2-25. The Spandan's proprietary algorithm has been successfully validated against the CTS (Conformance Testing Services) Database and CSE (Common Standards for Quantitative Electrocardiography) Database, mandated by IEC 60601-2-25. The Spandan pro ECG algorithm was described in a previous paper, where Spandan was used to validate Decisions for Percutaneous Coronary Intervention (PCI) (5). This case report aims to highlight the ability of a smartphone-based ECG (Spandan Pro) device to detect multivessel CAD and its diagnostic concordance with coronary angiography.

Case Description

A 51-year-old woman presented to the Swami Rama Himalayan University (SRHU) Hospital (Dehradun, India) on 20 February 2024, with complaints of chest pain. At the time of presentation, the patient did not exhibit symptoms such as shortness of breath, syncope, palpitations, or gastrointestinal symptoms. She had a medical history of CAD, hypertension (HTN), TVD, and a prior episode of acute coronary syndrome (ACS). She had undergone PCI with stent placement on 29 January 2023. The patient did not present with comorbid conditions such as diabetes mellitus, and her social history revealed that she was a non-smoker. The patient's anthropometric measurements revealed a height of 155 cm and a weight of 60 kg, resulting in a body mass index (BMI) of 24.97 kg/m^2, which is within the normal range.

The patient was compliant with her medications. Her medications included dual antiplatelet agents (aspirin and ticagrelor), atorvastatin (a high-potency statin used as lipid-lowering therapy), isosorbide nitrate (a long-acting nitrate), low molecular weight heparin, pantoprazole (a proton pump inhibitor), and metoprolol succinate (a beta-blocker frequently prescribed for CAD, HTN, and ACS). On physical examination, the patient was alert and oriented to time, person, and place. At the time of presentation, her blood pressure was 110/70 mmHg. She was clinically stable, with no signs of hemodynamic compromise.

The initial assessment of the patient involved a conventional 12-lead ECG (Philips PageWriter ECG) on 20/02/24 at 3:48 PM. The conventional 12-lead ECG showed significant ST-segment depression in lead AVF, along with T wave inversion in lead III. These findings can suggest myocardial ischemia. These findings were interpreted as non-specific but indicative of possible wall ischemia (Figure 1A). The next morning at 8:57 AM on 21/02/24, a comprehensive 12-lead ECG was recorded using the smartphone-based ECG device. The smartphone-based ECG detected ST-segment depression in multiple leads: V4, V5, V6, AVL, II, III, and AVF. This pattern was consistent with inferolateral ischemia, potentially involving the LAD and LCX arteries, suggesting double-vessel disease (DVD) (Figure 1B).

The same evening (21/02/24), diagnostic coronary angiography was performed at 4:30 PM to clarify the extent and severity of CAD. The angiogram revealed the following findings:

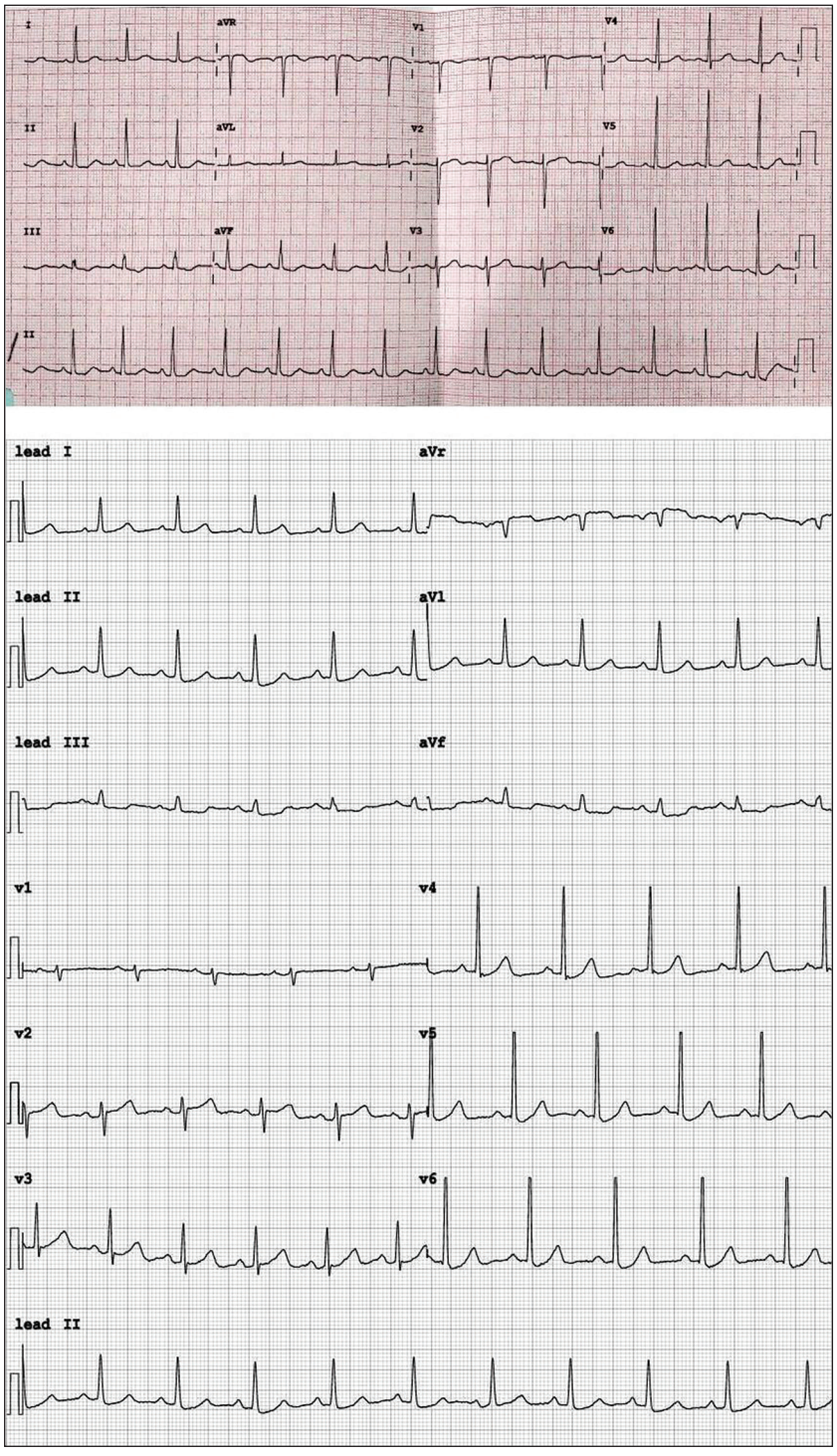

Figure 1. ECG report. (A) A conventional 12-lead ECG test report showing ST elevation of lead AVF and T wave inversion of lead III. (B) Spandan ECG test report showing ST-segment depression in leads V4, V5, V6, AVL, II, III, and AVF.

LMCA: Proximal 30-40% lesion, mid normal, distal 50% lesion. LAD: Type III artery, ostial 70% lesion, mid 70% lesion, distal normal. D1: Normal. LCX: Non-dominant, ostial 70% lesion, mid 90% lesion, distal normal. OM1: Normal. RCA/PDA: Dominant, proximal normal, mid 90% lesion.

The final impression of the coronary angiography confirmed TVD involving the LAD, LCX, and RCA, as well as additional involvement of the left main coronary artery (LMCA) (Figure 2). Based on the angiographic findings of TVD, the cardiology team recommended surgical vascularization through coronary artery bypass grafting (CABG) as the first approach. Percutaneous transluminal coronary angioplasty (PTCA) was advised as an alternative approach because of the nature and extent of the disease. Unfortunately, the patient was lost to follow-up after discharge, and further details are not available.

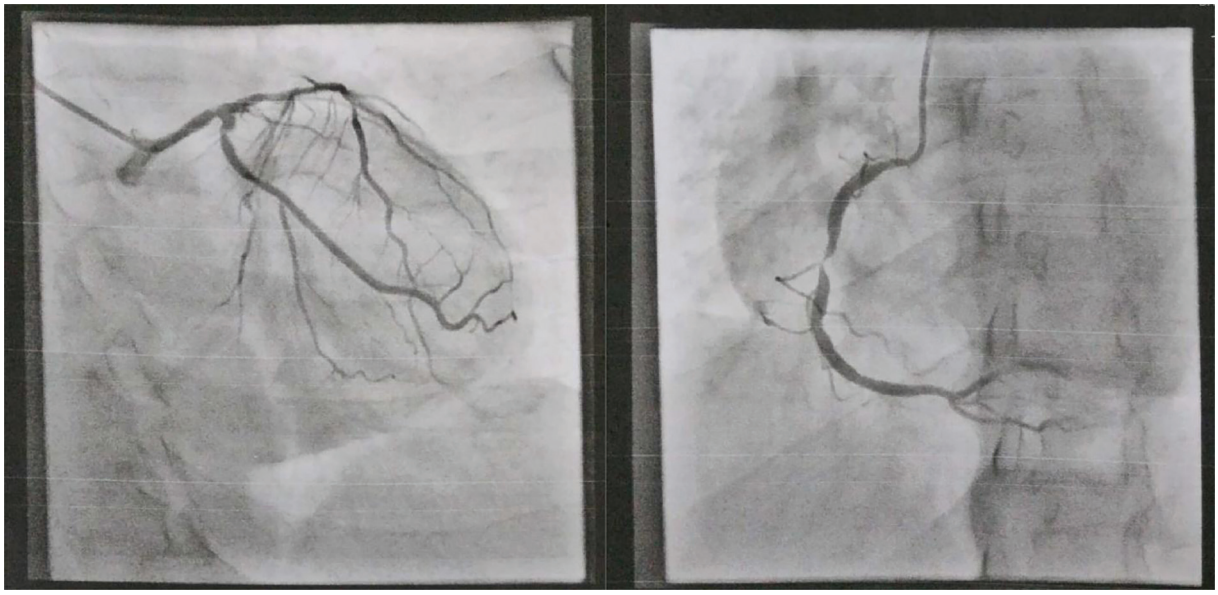

Figure 2. Coronary angiography report showing TVD involving the LAD, LCX, and RCA, with additional LMCA involvement.

Discussion

This case presents the diagnostic concordance between smartphone-based ECG and coronary angiography in detecting severe ischemic heart disease. Conventional 12-lead ECG results suggested myocardial ischemia. The smartphone-based ECG device identified ST depression in various leads, indicative of inferolateral ischemia and DVD possibly involving the LAD and LCX arteries. There was good concordance between these findings and the angiographic findings of TVD involving the LAD, LCX, and RCA, with additional LMCA involvement. Although coronary angiography remains the ultimate gold standard for identifying anatomical occlusions, the conventional 12-lead ECG remains a potent tool for predicting the culprit vessel and guiding early triage. This case illustrates that smartphone-based ECG can detect ischemic changes suggestive of vessel involvement. While it cannot replace the anatomical detail provided by angiography, it can at least identify significant ischemia and provide a preliminary indication of the territories that may be involved. Its portability, relatively low cost, and ease of use make it particularly valuable in ambulances, remote centers, or pre-hospital settings, enhancing early detection and supporting timely clinical decision-making.

An increasing number of studies support the use of smartphone-based ECGs to detect ischemia and arrhythmias, especially in ambulatory settings and resource-constrained locations. Mahajan et al. conducted a study to validate the accuracy of Spandan ECG interpretation in detecting ischemia compared to conventional 12-lead ECG interpretation. They reported a sensitivity of 87.4%, specificity of 97.4%, positive predictive value (PPV) of 87.4%, and Negative Predictive Value (NPV) of 89.85% (6). Another study used Spandan Pro ECG to evaluate its diagnostic efficacy for the detection of ST elevation against conventional 12-lead ECGs, thereby aiding cardiologists' decisions to perform PCI. The device showed excellent concordance with conventional 12-lead ECGs, particularly in leads II, III, and AVF, with Pearson correlation coefficients of almost 1. The ST elevations observed in the Spandan pro ECG devices did not show a significant statistical difference from those of the conventional 12-lead ECG. The device had 94% sensitivity and a 94% positive predictive value for ST-elevation detection, thus supporting its role in decision-making for PCI (5). Another study employed the Spandan pro ECG device to evaluate the

diagnostic performance of smartphone ECG devices in Cardiac Care Units and Cardiac Intensive Care Unit-admitted patients regarding the presence of STEMI or NSTEMI.The diagnosis was made by a team of cardiologists after a thorough examination of ECG records, which showed 100% specificity, 93% sensitivity, 80% negative predictive value, and 100% positive predictive value, yielding an F-score of 0.96 and a Matthews correlation coefficient of 0.86 (7). Studies highlighted the capability of the Spandan smartphone-based ECG in identifying myocardial ischemia. Similarly, in the study by Muhlestein et al., another ECG device (AliveCor™ Heart Monitor) was used, and the study confirmed the potential of smartphone ECGs for the evaluation of acute ischemia (8).

The patient in our study complained of chest pain, which is the most typical clinical manifestation of CAD. According to Chowdhary et al., 62.16% of patients experienced chest pain (9), and according to Haider et al., 96% of patients had chest pain (10). The patient in our study had a history of recognized HTN. According to Takieddin et al., 61.46% of patients had HTN (11), and according to Tsega et al., HTN was the most prevalent comorbid condition, occurring in 47.3% of patients (12). Patients with HTN may have an increased risk of MI and heart failure due to cardiac hypertrophy, and they may also contribute to the development of atherosclerosis through mechanical stress. Overall, HTN is one of the most common comorbidities in patients with CAD.

The availability of basic ECG remains low in many remote primary care settings in developing countries, including India. For patients presenting with chest pain, early identification of STEMI is crucial for timely stabilization and referral. A smartphone-based ECG enables the early and accurate detection of significant ischemia, allowing remote healthcare workers to initiate essential medications, coordinate urgent transport, and alert receiving hospitals in advance. This early identification can help avoid harmful delays in accessing appropriate care, which is particularly relevant in conditions such as myocardial ischemia. It also helps prioritize limited resources and reduces avoidable patient referrals by distinguishing non-life-threatening cases. Compared with other portable ECG devices, the Spandan platform leverages the smartphone's high-resolution display, provides advanced on-device algorithms for real-time interpretation of 12+ arrhythmias and 14+ types of myocardial infarction/ischemia, and supports a seamless digital workflow for instant report sharing and teleconsultation. Battery-free, highly portable, and paperless, it is easy to deploy in wards, triage areas, or community health camps. This case highlights the clinical utility of smartphone-based ECG in triaging chest pain and the early detection and risk assessment of patients with known cardiovascular risk factors, even outside tertiary centers. Despite these promising findings, this case report has several limitations. As this is a single case report, the generalizability of Spandan's diagnostic accuracy cannot be assumed without large-scale validation studies. Importantly, this case is part of an ongoing clinical trial with a substantial sample size, which will allow for a comprehensive assessment and confirmation of these preliminary observations.

Conclusion

In this case, smartphone-based ECG demonstrated ischemic changes suggestive of DVD, which were later confirmed as TVD with LMCA involvement on coronary angiography. This case report supports the use of smartphone-based ECG devices as a valuable adjunct to conventional 12-lead ECG for the early detection and risk assessment of patients with chest pain and known cardiovascular risk factors.

What Is Already Known on This Topic:

Early diagnosis of CAD is important for early intervention and better outcomes, particularly in patients who have experienced ACS or undergone PCI. Standard 12-lead ECG and coronary angiography are standard methods for assessing ischemia and vessel involvement. However, these modalities may not be readily available in resource-constrained or remote areas. Smartphone-derived ECG devices have shown promise in detecting arrhythmias and monitoring rhythms; however, their application in diagnosing ischemia and multivessel disease is still being explored.

What This Study Adds:

This case demonstrates the potential of a smartphone-based ECG device to detect inferolateral ischemia indicative of DVD, which was later confirmed as TVD through coronary angiography. This underscores the ability of portable ECG technology to aid in the early identification of multivessel coronary involvement, supporting its role in preliminary screening and triage, especially in patients with complex CAD.

Conflict of Interest: NC, DA, SYPS, and MP are currently employed by Sunfox Technologies. The other authors have no conflicts of interest.

Ethical Approval and Patient Consent: The patient described in this case report was enrolled in a broader clinical trial that received ethical approval from the Swami Rama Himalayan University Institutional Ethics Committee (Approval No. SRHU/HIMS/E-1/2024/07) with CTRI No. CTRI/2024/07/071055. Written informed consent was obtained from the patient for the publication of this study.

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Standard of Reporting: CARE guidelines and methodology were followed.

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Supplementary Table: Timeline of The Case Report

Date	Time	Event
29/01/2023	-	The patient underwent PCI with stent placement (the exact vessel stented was not recorded).
20/02/2024	3:48 PM	A conventional 12-lead ECG was performed following complaints of chest pain.
21/02/2024	8:57 AM	Spandan smartphone-based ECG was performed, which showed inferolateral ischemia potentially involving the LAD and LCX, with a probable diagnosis of DVD.
21/02/2024	4:30 PM	The patient underwent coronary angiography, which confirmed the presence of TVD along with LMCA involvement.
21/02/24	-	Post-angiography, the patient was recommended for CABG as the first option and PTCA as an alternative option.

Case Report and Review of Literature
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B2 Thymoma with Intracardiac Extension Presenting as Superior Vena Cava Syndrome: Case Report and Literature Review

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Abstract

Objective. This article aims to emphasize the importance of considering invasive thymoma in the differential diagnosis of mediastinal masses and highlights the critical role of timely surgical and oncological management in improving patient outcomes. **Case Report.** We present the case of a 70-year-old woman who presented with signs of superior vena cava syndrome, including dyspnea, facial swelling, and fatigue. Advanced imaging and intraoperative findings revealed a large anterior mediastinal mass infiltrating the left brachiocephalic vein and superior vena cava, extending into both the right atrium and right ventricle. Surgical intervention was performed, and histopathological analysis confirmed B2 thymoma with a high Ki-67 proliferation index. Despite surgical intervention, the patient's condition deteriorated, and she ultimately succumbed to the disease. **Conclusion.** To the best of our knowledge, this is the first reported Bosnian case of B2 thymoma invading the brachiocephalic vein and superior vena cava and infiltrating both the right atrium and ventricle, causing superior vena cava syndrome. Despite their rarity, thymomas should always be considered in patients presenting with an enlarged mediastinum.

Key Words: B2 Thymoma ▪ Mediastinal Mass ▪ Superior Vena Cava Syndrome ▪ Echocardiography.

Introduction

Invasive thymoma extending into the brachiocephalic vein, superior vena cava (SVC), right atrium (RA), and right ventricle (RV) is a very rare but clinically significant condition. The incidence of thymoma is relatively low, with approximately 0.17 cases per 100,000 individuals globally (1). Invasive thymomas account for approximately 30% of all thymomas and can invade mediastinal organs, such as the pleura and pericardium (2). However, only a small number of thymomas are associated with SVC syndrome, often due to extrinsic compression rather than intravascular invasion. Intravascular invasion of the SVC extending into the RA and RV is an even rarer phenomenon, first reported by Suzuki et al. in 1976, with few cases documented since then (3-5). The main objective of this case report is to describe a rare manifestation of type B2 thymoma with simultaneous SVC and intracardiac invasion and to place its presentation within the current clinical and pathological context while highlighting its diagnostic and prognostic implications.

Case Reports

Clinical Presentation

A 70-year-old woman was admitted with a several-month history of difficulty in breathing, swelling of the face and neck, and excessive fatigue. She reported that her symptoms had gradually worsened, interfering with her daily activities, and had

become more acute in the past few days. Physical examination revealed facial plethora, distension of the jugular veins, and bilateral lower limb edema, consistent with impaired venous return. Auscultation revealed diminished breath sounds over the left hemithorax.

Imaging Findings and Surgical Approach

An initial chest X-ray, obtained during pulmonary evaluation, showed widening of the superior mediastinum, an enlarged cardiac silhouette, and a left-sided pleural effusion. As part of further cardiac work-up, transthoracic echocardiography was performed and revealed a large (8.5 cm × 4 cm) echogenic mass within the right atrium, prolapsing into the right ventricle during diastole, which was initially interpreted as a possible atrial myxoma. Owing to the urgent clinical presentation and high suspicion of an intracardiac tumor, the patient was referred directly for cardiovascular surgery without preoperative cross-sectional imaging. The patient underwent median sternotomy on December 14, 2023, with extirpation of the tumor formation involving the right atrium, right ventricle, and superior vena cava. Intraoperatively, a solid tumor formation was identified in the anterior mediastinum at the thymic site, infiltrating the wall of the left brachiocephalic vein and extending into the superior vena cava, right atrium, and right ventricle. Complete excision was not feasible because of vascular invasion, and the tumor was removed in fragments. The postoperative course was uneventful and without complications. Following surgery, contrast-enhanced computed tomography (CT) of the chest was performed for staging and postoperative assessment. It revealed a large residual anterior mediastinal mass, measuring approximately 10.3×5.2×8 cm with irregular margins and central necrosis (Figure 1).

The mass continued to infiltrate the wall of the left brachiocephalic vein and superior vena cava (SVC). In addition, multiple enlarged mediastinal lymph nodes were present, particularly in the pretracheal and aortopulmonary window regions, along with pathologic lymphadenopathy in the left axilla and upper abdomen (Figure 2). Free peritoneal fluid was noted around the liver, spleen, and within the pelvis, consistent with advanced systemic disease.

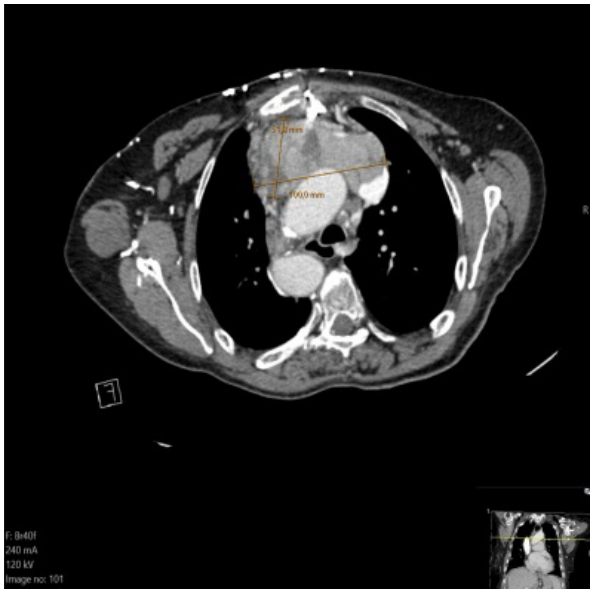

Figure 1. Contrast-enhanced CT of the chest showing a large anterior mediastinal mass infiltrating the left brachiocephalic vein.

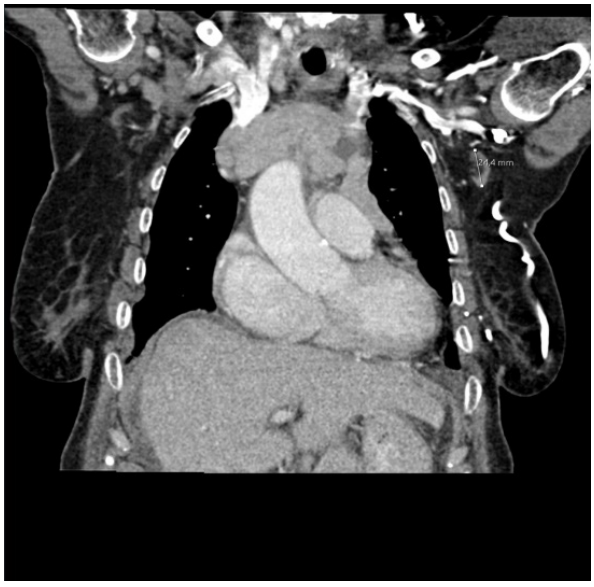

Figure 2. Contrast-enhanced CT demonstrating pathological lymphadenopathy: enlarged lymph node in the left axilla.

Pathohistological Findings

Pathohistological analysis of the resected tumor fragments revealed tumor tissue with a focally present connective capsule on the surface. The tumor is composed of smaller and larger nodules, as well as areas formed by tumor cells. Approximately 75% of these cells are characteristic of immature thymocytes, immunohistochemically positive for CD3, CD5, bcl2, TdT, CD1a (weakly positive), and CD99. Interspersed among these are another population of cells with epithelioid characteristics, displaying oval to polygonal, occasionally slightly elongated nuclei with finely dispersed chromatin and focally visible nucleoli. These cells are immunohistochemically positive for AE1/AE3 and p63. Acellular loose vascularized connective tissue can be seen between the tumor cells. Immunohistochemically, the tumor cells are negative for CD20, bcl6, synaptophysin, chromogranin, CD56, TTF1, and CD10. The Ki67 proliferative activity index is almost 100%. The morphological and immunohistochemical characteristics of the analyzed sections support a diagnosis of thymoma, B2 type. Because the specimen was received in fragments, the surgical margin status and pT stage could not be determined.

Outcome

Following surgery and further testing, the patient initially recovered without perioperative complications and was discharged in stable condition. She was subsequently evaluated by a multidisciplinary oncology board, which included specialists in medical oncology, radiation oncology, surgery, clinical pharmacology, and pathology. Given the extent of the disease and systemic involvement, she was deemed unsuitable for curative oncologic treatment and was referred for palliative management. Over the following weeks, her condition progressively deteriorated with worsening fatigue and edema. After several refusals, the patient was finally admitted to the Center for Palliative Care. Despite supportive therapy, her condition progressively deteriorated, and she passed away approximately three months after diagnosis.

Discussion

Thymomas are the most common primary tumors of the anterior mediastinum, although their overall incidence remains very low, estimated at 0.17 cases per 100,000 population annually (1). They are frequently detected incidentally or present with nonspecific thoracic symptoms, such as cough, chest pain, or dyspnea, caused by local compressive effects (2, 6, 7). In addition to local compressive symptoms, thymomas are notable for their frequent association with paraneoplastic syndromes, most prominently myasthenia gravis, which occurs in 30–50% of patients. Other autoimmune conditions, including pure red cell aplasia and hypogammaglobulinemia, may also be seen, although they are less common (8). Therefore, patients may present with a spectrum ranging from asymptomatic incidentalomas to chest symptoms and systemic autoimmune manifestations (9).

Radiologically, thymomas typically appear as well-circumscribed lobulated soft-tissue masses in the anterior mediastinum on chest CT or MRI (10). On CT, smaller tumors usually demonstrate homogeneous soft-tissue density, whereas larger lesions may contain areas of necrosis, cystic change, or calcification (11). MRI can further provide tumor composition, with thymomas often demonstrating signal intensity similar to or higher than muscle on T1-weighted sequences and higher than muscle on T2-weighted sequences, sometimes approaching that of fat, which may hinder distinction from surrounding mediastinal fat (12). Features suggesting invasive disease include irregular or ill-defined borders, loss of normal fat planes between the tumor and adjacent structures, vascular encasement, and pleural implants (13).

According to the 2021 WHO classification, thymomas are divided into types A, AB, B1, B2, and B3 based on the morphology of epithelial cells and the proportion of lymphocytes (14). Among the histological subtypes, type B2 thymoma accounts for approximately 20% of all thymomas. It is composed of scattered epithelial cells with vesicular nuclei and prominent nucleoli within a dense lymphocyte population (15). Type B2 thymomas

exhibit moderate aggressiveness, with reported five-year survival rates between 60% and 70% (12). Their prognosis is worse than that of types A, AB, and B1, but more favorable than that of type B3 or thymic carcinoma (16).

Invasive thymomas typically extend to adjacent mediastinal structures, such as the pleura, pericardium, or lungs (17). True intravascular or intracardiac growth is extremely rare. Since Suzuki et al. first described a thymoma with intravascular extension into the superior vena cava (SVC) and right atrium in 1976 (3), only a small number of such cases have been reported. In most instances, cardiac involvement is limited to pericardial invasion rather than direct intracaval or intracardiac extension (18). The presumed mechanism of intracaval spread involves the invasion of small thymic veins with subsequent downstream expansion into larger vessels and cardiac chambers (19).

Our case illustrates several unusual and clinically significant features. The patient presented with the classical signs of SVC syndrome, including dyspnea, facial swelling, and venous distension, resulting from tumor invasion of the left brachiocephalic vein and SVC. The tumor further extended into the right atrium and right ventricle, prolapsing through the tricuspid valve during the cardiac cycle, an exceptionally rare manifestation. Pathohistological analysis confirmed a type B2 thymoma with an almost 100% Ki-67 proliferation index, reflecting highly aggressive tumor behavior. Finally, the initial echocardiographic interpretation suggested a right atrial myxoma, highlighting the diagnostic challenges when invasive mediastinal tumors mimic primary intracardiac masses.

The therapeutic implications are equally important. Complete surgical resection remains the key treatment for thymoma and is associated with better long-term survival outcomes (20). In our case, however, en bloc resection was not feasible due to extensive vascular and intracardiac invasion, and the tumor was removed in fragments. Postoperative multidisciplinary evaluation concluded that the patient was not a candidate for further oncologic therapy, highlighting the limited treatment options available in such advanced stages. Reported outcomes of similar cases confirm the poor prognosis of thymomas with intracaval or intracardiac spread, despite surgical intervention (21).

Based on six well-documented cases with dual-chamber (RA + RV) invasion identified in the literature, together with the present case, a consistent clinicopathological pattern emerged, as shown in Table 1. All patients presented with manifestations of superior vena cava obstruction, such as dyspnea, facial and upper limb swelling, and distended neck veins (Table 1). Imaging confirmed a continuous tumor extension from the mediastinum into the right atrium and, in some cases, across the tricuspid valve into the right ventricle, indicating direct venous propagation rather than hematogenous spread. From a treatment perspective, complete surgical resection with cardiopulmonary bypass was associated with better outcomes compared to chemotherapy or radiotherapy alone (Table 1). The almost universal involvement of the right atrium and the rarity of right ventricular extension likely reflect the venous anatomy of the thymus. Thymic veins predominantly drain into the brachiocephalic veins and superior vena cava, providing a direct pathway for tumor spread into the right atrium (22). In contrast, right ventricular involvement requires additional progression across the tricuspid valve, which is quite unusual and an anatomically unfavorable pathway for tumor expansion. The histological spectrum of these cases ranged from type A to type B2 thymomas, including spindle-cell and mixed epithelial forms, without a clear correlation between subtype and invasive potential (Table 1). The present case of a B2 thymoma with a nearly 100% Ki-67 proliferation index illustrates how high proliferative activity may promote aggressive intraluminal growth beyond the right atrium.

Surgical resection under cardiopulmonary bypass (CPB) was performed in four of the seven patients, allowing partial or complete tumor removal. Three of these patients were alive at follow-up, whereas non-surgical management resulted in poor outcomes (Table 1). These findings emphasize that CPB-assisted resection with possible

Table 1. Reported Cases of Thymoma With Dual-Chamber (RA + RV) Intracardiac Invasion

Year	First author	Histology (WHO)	Intracardiac involvement	Extra-cardiac spread	Clinical presentation	Treatment	Outcome at reporting
1990	Airan (4)	Malignant thymoma (spindle-cell/ epithelial type)	RA + RV	SVC, right lung, aortic arch, tracheal bifurcation (compression)	SVC syndrome (neck and chest vein distension, hepatomegaly, ascites, weight loss)	Partial resection (CPB, RA + RV) + postoperative RT	Alive
2000	Hayashi (23)	Mixed (predominantly epithelial) thymoma	RA + RV	LBCV, SVC	SVC syndrome, dyspnea	Two resections under CPB + mediastinal RT	Alive
2003	Funakoshi (24)	Type A thymoma; Masaoka stage IVb	RA+RV	SVC, Bilateral BCV	Facial and left upper-limb swelling (SVC syndrome)	Urgent radical resection with SVC reconstruction + postoperative RT	Alive
2016	Chadha (25)	Invasive thymoma (histological type not specified)	RA + RV	SVC, Anterior mediastinum	Dyspnea, chest pain	Patient declined surgical treatment	Alive
2016	Senanayake (26)	Thymoma (histological type not specified)	RA + RV	SVC, Bilateral BCV	Exertional dyspnea, peripheral edema, bilateral pleural effusions (SVC syndrome)	Chemotherapy (cisplatin-based), palliative RT planned	Deceased before RT, 6 months after presentation
2022	Asami-Noyama (27)	Type A thymoma	RA + RV	Bilateral BCV, SVC, lung, lymph nodes	Facial and upper-limb edema, dyspnea, SVC syndrome	Carboplatin + paclitaxel chemotherapy; sudden obstructive shock; anticoagulation attempted	Deceased 5 days after start of chemo (massive intracardiac thrombosis)
2025	Present case	B2 thymoma	RA + RV	LBCV, SVC	SVC syndrome, facial swelling, dyspnea	Partial resection (fragmented)	Deceased

RA=Right atrium; RV=Right ventricle; SVC=Superior vena cava; CPB=Cardiopulmonary bypass; LBCV=Left brachiocephalic vein; RT=Radiotherapy; BCV=Brachiocephalic veins.

venous reconstruction provides the best chance of survival when anatomically feasible. Our patient exhibited the most extensive pattern of invasion, involving the left brachiocephalic vein, SVC, right atrium, and ventricle. Despite partial resection, the outcome was unfavorable, consistent with other reports of incomplete excision. Collectively, this focused analysis of seven RA + RV cases provides a clearer understanding of this rare disease behavior, underscoring the need for early imaging-based diagnosis and multidisciplinary planning. While histology alone does not predict invasion, the extent of venous involvement and the possibility of complete resection remain the main determinants of prognosis.

In summary, this case highlights the importance of including thymoma in the differential diagnosis of mediastinal masses presenting with SVC syndrome. It emphasizes the limitations of echocardiography in differentiating invasive mediastinal tumors from intracardiac lesions, and the need for comprehensive imaging and pathohistological confirmation. To our knowledge, this is the first Bosnian case of type B2 thymoma with simultaneous invasion of the brachiocephalic vein, SVC, right atrium, and right ventricle. Recognition of this rare growth pattern is critical for timely diagnosis and multidisciplinary management, although therapeutic options and outcomes remain limited in advanced disease.

Conclusion

Intracardiac extension of thymoma is rare but carries significant diagnostic and therapeutic challenges. This case adds to the limited global literature and represents, to the best of our knowledge, the first regional report of a B2 thymoma invading both the right atrium and ventricle. Recognition of this unusual growth pattern is important for timely diagnosis, surgical planning, and coordinated, multidisciplinary care.

What Is Already Known on This Topic:
Thymomas are the most prevalent primary mediastinal malignancy, but they are rare and often exhibit local invasion into surrounding structures. "Transvenous" cardiac metastases, or intracaval growth extending into the right atrium, rarely occur, and only a few examples of transcaval extension with intracardiac involvement have been documented.

What This Study Adds:
In contrast to previous studies, where the majority of thymomas with cardiac involvement were restricted to the right atrium, pericardium, or SVC, we present a case of thymoma metastasis to both right heart chambers with atypical clinical manifestations.

Authors' Contributions: Conception and design: AM and SU; Acquisition, analysis and interpretation of data AM, HO and EM; Drafting the article: AK, AM and FS; Revising it critically for important intellectual content: AM and AM; Approved final version of the manuscript: AM, SU, HO, EM, FS, AK and AM.

Conflict of Interest: The authors declare that they have no conflict of interest.

Informed Consent: Approval was obtained from the Ethical Committee of the University Clinical Center Tuzla for this case report. Date of approval: February 26, 2025. Approval number: 02-09/2-206-2/24.

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