The Prognostic Role of Diagnostic Criteria for COVID-19 Associated Pulmonary Aspergillosis: A Cross-Sectional Retrospective Study

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Article

The Prognostic Role of Diagnostic Criteria for COVID-19 Associated Pulmonary Aspergillosis: A Cross-Sectional Retrospective Study

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Abstract: Several criteria exist to diagnose pulmonary aspergillosis with varying degrees of certainty in specific populations, including oncohaematological patients (EORTC/MSG), ICU patients (AspICU) and Covid-19 patients (ECMM). At the beginning of the pandemic, however, the diagnosis of Covid-19 Associated Pulmonary Aspergillosis (CAPA) could not be performed easily, and the decision to treat (DTT) was empirical. In this cross-sectional retrospective study including patients with SARS-CoV-2 infection and a suspicion of CAPA, we studied the concordance between the DTT and the three diagnostic criteria using Cohen's coefficient, then we identified the factors associated with the DTT and we corrected by treatment to study the influence of the diagnostic criteria on survival. We showed good concordance of DTT and AspICU and ECMM criteria, with "compatible signs", "positive culture" and "positive galattomannan" influencing the DTT. Treatment also showed a positive effect on survival once corrected for a putative, possible or probable diagnosis of CAPA using AspICU and ECMM criteria. We conclude that EORTC/MSGERC are not considered applicable in clinical practice due to lack of inclusion of signs and symptoms and do not lead to improved survival. AspICU and ECMM criteria showed a good degree of agreement with the DTT and a positive correlation with patient recovery.

Keywords: COVID-19; pulmonary aspergillosis; case definitions; CAPA; certain/probable/possible diagnosis

1. Introduction

COVID-19 Associated Pulmonary Aspergillosis (CAPA) is an opportunistic infection associated with severe COVID-19. It was recognised as a clinical entity soon after the beginning of the pandemic, with wildly varying incidence and prevalence depending on the diagnostic criteria (5,7%-27,7%)[1-4].

Specific criteria for the diagnosis of invasive aspergillosis exist since 2002, when the first European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria were compiled. The original article and its revisions propose three levels of certainty in the diagnosis of invasive aspergillosis (proven, probable and possible), and their intended use is limited to research, not clinical practice [5-7]. Moreover, they focus on immunosuppressed patients (solid organ and haematopoietic stem cell transplant recipients, solid cancer and haematological patients), with only the "proven" category being applicable to everyone, and thus their applicability to intensive care unit (ICU) and COVID-19 patients is limited [8].

Since then, efforts have been made to either expand these criteria to ICU patients [8] or to create new ones, like the AspICU algorithm [9], which has a more clinical focus and includes critically ill patients. The AspICU criteria distinguishes patients in proven, putative pulmonary aspergillosis and

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colonisation. Later, Schauwvlieghe *et al.* modified the criteria excluding the host factors (neutropenia, oncohematological malignancy, glucocorticoid treatment and congenital or acquired immunosuppression) [10]. Their aim was avoiding the automatic exclusion from a diagnosis of critically ill patients with influenza, who rarely have host factors but are nonetheless at high risk of aspergillosis [11].

Finally, the European Confederation of Medical Mycology (ECMM) provided criteria specific for COVID-19 patients in 2020 [12]. The ECMM criteria are specifically targeted at both research and clinical guidance and were derived from EORTC/MSG and AspICU criteria. SARS-CoV-2 positivity and need for ICU were considered entry criteria, and aspergillosis diagnosis was divided in proven, probable and possible.

Especially at the beginning of the pandemic, when diagnostic criteria were not available and procedures like lung biopsy and bronchoalveolar lavage (BAL) were limited, the decision to treat (DTT) was often empirically based. Thus, the main aim of our study is to retrospectively assess the concordance between DTT and the three criteria and to identify which of their factors are associated with the DTT. We then correct by treatment to study the influence of the diagnostic criteria on survival.

2. Results

2.1. Population

As described in Table 1, we retrospectively collected 196 patients with COVID-19 and a suspicion of CAPA. Median age was 64 years old (53-71), males were 139 and females 57. Median length of hospital stay was 26.1 days (14.3-49.0). Death was recorded for 66 patients (33.7%). ICU admission was recorded for 52 patients (44.8%) and mechanical ventilation (invasive and noninvasive) was needed for 65 patients (57.0%).

Table 1. Population description. Continuous data are presented with median and interquartile ranges (1°-3° quartile), categorical data with frequency and percentages. ICU: intensive care unit; CPAP: continuous positive air pressure; BAL: Bronchoalveolar lavage.

	Hospitalisation registry (n=196)
Age [years]	64 (53 – 71)
Sex	
Male	139 (70.9)
Female	57 (29.1)
Outcome	
Deceased	66 (33.7)
Discharged	130 (66.3)
Lenght of stay [days]	26.1 (14.3-49.0)
ICU admission	52 (44.8)
Mechanical ventilation	
CPAP	18 (15.8)

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Invasive	47 (41.2)
Comorbidities	
Diabetes	20 (17.7)
Obesity	11 (9.7)
Solid tumour	13 (11.6)
Chronic Obstructive Pulmonary Disease	24 (21.2)
AspICU and ECMM factors	
Compatible signs	83 (74.1)
Neutropenia: $<0.5*10^9$ [neu/L] before or at ICU admission	7 (6.1)
Cytotoxic agents	16 (14.3)
Steroids: 20 mg/day	98 (87.5)
Immunodeficiency	11 (9.7)
EORTC/MSG host factors	
Neutropenia: $<0.5*10^9$ [neu/L] for >10 days	7 (6.0)
Haematological malignancy	25 (21.7)
Hematopoietic stem-cell transplantation	11 (9.7)
Solid organ transplant	9 (7.8)
Steroids: 0.3mg/kg/day for >3 weeks	37 (32.6)
T-immunosuppressant	12 (10.6)
Treatment with B-cell suppressors	4 (3.5)
Acute graft-versus-host disease	1 (0.9)
Inherited severe immunodeficiency	1 (0.9)
Radiological pattern	
Atypical	88 (83.8)
Normal	12 (11.4)
Typical	5 (4.8)
Galattomannan antigen	
Material	

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BAL	80 (42.6)
Serum	108 (57.4)
Positivity	28 (14.8)
Median value	0.2 (0.2 – 2)
Cultures	
Material	
Bronchonasopharyngeal aspirate	6 (5.5)
Sputum	10 (9.3)
Induced sputum	1 (0.9)
Blood	1 (0.9)
BAL	90 (83.3)
Species	
Aspergillus	17 (15.2)
Candida	58 (51.8)
Aspergillus+Candida	17 (15.2)
AspICU classification	
Certain	0 (0.0)
Putative	20 (18.0)
Colonisation	2 (1.8)
ECMM classification	
Certain	0 (0.0)
Probable	19 (17.1)
Possible	2 (1.8)
EORTC/MSG classification	
Certain	0 (0.0)
Probable	10 (8.9)
Possible	27 (24.1)
Treatment	
Decision to treat	33 (16.8)

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Drug	
Echinocandin	3 (6.5)
Fluconazole	2 (4.3)
Voriconazole	24 (12.2)
Isavuconazole	2 (4.3)
Voriconazolo+amphotericin B	1 (2.2)

2.1.1. AspICU and ECMM criteria

According to the AspICU criteria, aspergillosis was certain in 0% of patients and putative in 18.0%. Colonisation was diagnosed in 1.8% of patients.

According to the ECMM criteria, aspergillosis was certain in 0% of patients, probable in 17.1% and possible in 1.8%.

The most prevalent host factors (applied as described in paragraph 4.4 and Table 1) were "use of steroids" (87.5%) and "presence of compatible signs" (74.1%).

2.1.2. EORTC/MSG criteria

According to the criteria, aspergillosis was certain in 0% of patients, probable in 9.0% and possible in 24.3%. The most prevalent host factor was use of steroids (32.7%).

2.1.3. Common factors and treatment

The main radiological pattern was atypical (83.8%) and galattomannan positivity was achieved in only 14.8% of patients. Aspergillus was cultured in 34 patients (30.4%). The DTT was applied in 33 patients (16.8%), mainly with voriconazole alone (24 patients, 12.2%).

2.2. Concordance of diagnostic criteria with the decision to treat

The 2x2 tables featuring the DTT and each of the three diagnostic criteria are shown in Table 2. Concordance among the DTT and the criteria is shown in Table 3. Cohen's κ coefficient is 0.46 and 0.44 for AspICU and ECMM criteria respectively, showing good concordance with the DTT ($p<0.001$ for both analyses), while it is only 0.16 for EORTC/MSG criteria ($p>0.05$). A further comparison between AspICU and ECMM criteria showed almost complete concordance ($\kappa=0.91$, $p<0.001$).

Table 2. 2x2 tables showing the relationship between the decision to treat (DTT) and the classification according to AspICU, ECMM and EORTC/MSG diagnostic criteria, simplified to binary criteria (patients to whom criteria are not applicable or are colonised, patients who have or might have pulmonary aspergillosis).

AspICU			
		Colonisation/not applicable	Certain/putative
Treatment	No	74	4
	Yes	16	13
ECMM			

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		Not applicable	Certain/probable/possible
Treatment	No	73	5
	Yes	16	13
EORTC/MSG			
		Not applicable	Certain/probable/possible
Treatment	No	57	22
	Yes	16	13

Table 3. Concordance between DTT and AspICU, ECMM and EORTC/MSG criteria expressed as Cohen's κ coefficient. Green represents a concordance value with p<0.001, red represents a concordance value with p>0.05.

Concordance (expressed as Cohen's κ coefficient)				
	DTT	AspICU	ECMM	EORTC/MSG
DTT		0.46	0.44	0.16
AspICU			0.91	0.02
ECMM				0.00
EORTC/MSG				

Factors influencing the decision to treat

The logistic regression on criteria and their factors influencing the DTT was performed on 112 patients (who had available data on criteria factors and on treatment). The univariate analysis showed significant influence of factors "compatible signs" and "positive galattomannan" (Table 4).

Table 4. Univariate analysis showing correlation between the criteria's factors and the decision to treat. DTT: decision to treat; ICU: intensive care unit.

Factors	**DTT yes (n=31)**	**DTT no (n=81)**	**P value**
Neutropenia: <0.5*10^9 [neu/L] for >10 days	3 (9.7)	4 (4.9)	0.39
Neutropenia: <0.5*10^9 [neu/L] before or at ICU admission	2 (6.5)	5 (6.2)	1

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Haematological malignancy	8 (25.8)	16 (19.8)	0.66
Hematopoietic stem-cell transplantation	5 (16.1)	6 (7.4)	0.17
Solid organ transplant	2 (6.5)	7 (8.6)	1
Steroids: 0.3mg/kg/day for >3 weeks	11 (35.5)	25 (32.1)	0.82
Steroids: 20mg/day	26 (90.3)	69 (87.3)	0.91
T-immunosuppressant	5 (16.1)	7 (8.6)	0.42
B-immunosuppressant	-	4 (4.9)	0.57
Acute graft-versus-host disease	-	1 (1.2)	1
Inherited severe immunodeficiency	1 (3.2)	-	0.27
Compatible signs	28 (90.3)	54 (67.5)	**0.03**
Cytotoxic agents	3 (10.0)	13 (16.2)	0.55
Immunodeficiency	2 (6.5)	9 (11.1)	0.72
Atypical Radiologic pattern	5 (16.7)	12 (16.7)	1
Galactomannan positivity	10 (32.2)	7 (8.6)	**<0.001**
Positive culture of Aspergillus	17 (63.0)	25 (56.8)	0.79

2.3. Correlation between survival and diagnostic criteria

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The logistic regression on criteria and their factors influencing survival was performed on 112 patients. The univariate analysis (Table 5) showed a significant association of mortality with cytotoxic agents and B-cell suppressants therapy, and of survival with antifungal treatment. Moreover, as shown in Table 6, when adjusted for the three diagnostic criteria (and cytotoxic agents and B-cell suppressants), treatment showed a significant association with survival especially when using AspICU and ECMM criteria (OR 6.17 and 5.36 respectively, $p<0.05$) but not for EORTC/MSG (OR 3.80, $p=0.05$).

Table 5. Univariate analysis showing correlation between survival and the diagnostic criteria's factors. NA: not available; ICU: intensive care unit.

Factors	Survivors (n=86)	Deceased (n=26)	P value
Neutropenia: $<0.5*10^9$ [neu/L] for >10 days	5 (5.8)	2 (7.7)	0.66
Neutropenia: $<0.5*10^9$ [neu/L] before or at ICU admission	5 (5.8)	2 (7.7)	0.66
Haematological malignancy	16 (18.6)	8 (30.8)	0.29
Hematopoietic stem-cell transplantation	8 (9.3)	3 (11.5)	0.71
Solid organ transplant	8 (9.3)	1 (3.8)	0.68
Steroids: 0.3mg/kg/day for >3 weeks	26 (30.2)	11 (42.3)	0.36
Steroids: 20mg/day	75 (87.2)	22 (91.7)	0.81
T-immunosuppressant	11 (12.8)	1 (3.8)	0.29
B-immunosuppressant	1 (1.2)	3 (11.5)	**0.04**
Acute graft-versus-host disease	1 (1.2)	-	1
Inherited severe immunodeficiency	1 (1.2)	-	1
Compatible signs	63 (73.3)	19 (76.0)	0.99
Cytotoxic agents	7 (8.2)	9 (34.6)	**0.002**
Immunodeficiency	7 (8.1)	4 (15.4)	0.28
Atypical Radiologic pattern	16 (20.0)	1 (4.5)	0.11
Galactomannan positivity	12 (14.0)	5 (19.2)	0.73
Positive culture of Aspergillus	30 (55.6)	12 (70.6)	0.41
Treatment	28 (32.6)	12 (70.6)	**0.04**
AspICU	14 (16.3)	6 (24.0)	0.56
ECMM	15 (17.4)	6 (24.0)	0.65

 doi:10.20944/preprints202401.0062.v1

EORTC/MSG	25 (29.1)	12 (46.2)	0.16

Table 6. Multivariate analysis showing correlation between survival and diagnostic criteria. OR: odds ratio.

	OR	**95% confidence interval**	**P value**
All criteria			
Cytotoxic agents	0.18	0.04 – 0.76	**0.019**
B-cell suppressants	0.66	0.03 - 8.59	0.76
Treatment	7.12	1.67 - 43.71	**0.016**
AspICU	0.42	0.01 - 7.04	0.564
ECMM	0.45	0.03 - 13.95	0.583
EORTC/MSG	0.53	0.18 - 1.59	0.243
AspICU			
Cytotoxic agents	0.17	0.04 - 0.62	**0.008**
B-cell suppressants	0.58	0.02-7.03	0.687
Treatment	6.17	1.49 - 36.81	**0.023**
AspICU	0.22	0.05 - 0.89	**0.035**
ECMM			
Cytotoxic agents	0.14	0.03 - 0.57	**0.006**
B-cell suppressants	0.64	0.03 - 7.80	0.738
Treatment	5.36	1.40 - 28.33	**0.025**
ECMM	0.23	0.06 - 0.92	**0.036**
EORTC/MSG			
Cytotoxic agents	0.25	0.06 - 0.97	**0.042**
B-cell suppressants	0.48	0.03 - 5.33	0.572
Treatment	3.80	1.10 - 18.16	0.054
EORTC	0.61	0.22 - 1.76	0.343

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3. Discussion

In our cohort of 196 patients, we found a prevalence of probable/possible/putative CAPA of 8.9-24.1% according to the criteria. No certain cases of CAPA were found; we attribute this to the difficulties in performing invasive procedures such as lung biopsy during the pandemic and in critically ill patients.

We found a good agreement between the physicians' DTT and the classification of patients according to AspICU and ECMM diagnostic criteria. The factors who lead to the DTT were "presence of compatible signs" and "positive galattomannan".

Both "compatible signs" and "positive galattomannan" are used by the AspICU and ECMM criteria but not by EORTC/MSG criteria, that, in fact, did not show concordance with the DTT. Moreover, the AspICU and ECMM criteria showed almost perfect concordance between them.

Concerning survival, treatment showed a positive effect that was confirmed once corrected for a putative, possible or probable diagnosis of CAPA using AspICU and ECMM criteria, but not using EORTC/MSG.

The inappropriateness of the EORTC/MSG criteria for the diagnosis of CAPA has been suspected since the start of the pandemic. This was reasonable, since the EORTC/MSG criteria were developed specifically for oncohaematological patients while the AspICU and ECMM criteria were destined to ICU and COVID-19 patients. This inappropriateness has been confirmed also by the only other study we could find that compared the criteria for the diagnosis of CAPA [13]. The study was conducted in Germany on 684 critically ill patients and compared EORTC/MSG and modified AspICU criteria, finding a Cohen's κ of 0.14, which is comparable to our finding. Unfortunately, the analysis did not include ECMM criteria. The concordance between AspICU and ECMM criteria was less expected. In fact, the first reports of CAPA based on AspICU or modified AspICU criteria led to an overestimation of its prevalence (up to 30%) [14-15], and even though larger studies brought down this number to 3.8% [16] they still advocated for a low threshold of suspicion, with the consequent risk of overtreatment. The development of the ECMM criteria had the effect of reducing the prevalence despite being based mostly on expert opinion and low grade evidence [17] but variable incidence is still reported [2, 18]. Our work showed that the choice of starting a treatment does not rely on those factors that differ between the two criteria (the host factors) but on clinical presentation and microbiological factors. This restricts the differences in management that would derive from the use of one diagnostic criterion over the other. This is further confirmed by our findings on survival: if treatment had been based on AspICU or ECMM classification, it would have led to improved odds of survival ($OR>5$, $p<0.05$). Use of EORTC/MSG, on the other hand, would have still led to improved survival, with the OR being 3.80 and the lower limit of the confidence interval being >1, but it did not reach statistical significance.

We did not find any literature on the effect of criteria on the DTT or their correlation to survival, although a Belgian study conducted ICU patients found that, when CAPA is diagnosed with ECMM criteria, the additional presence of EORTC/MSG host factors leads to increased mortality [19].

Our study presents several limitations: apart from its single-centre and retrospective nature, the risk of selection bias is apparent in our assumption that patients with a suspicion of CAPA would have undergone at least one galatomannan antigen test. Another bias is our definition of a patient requiring the ICU, but since this study includes the first waves of the pandemic, where ICU beds where scarcely available and ordinary wards functioned as ICU units [20], we believe that this did not overly affect the results.

Prospective international studies on the impact of diagnostic criteria on accuracy and patients' outcome should be planned.

4. Materials and Methods

4.1. Study design and setting

We conducted a cross-sectional retrospective study including patients admitted to Fondazione IRCCS Policlinico San Matteo of Pavia from the 21st of February 2020 to 30th of April 2022. All

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patients were hospitalised for a PCR-proven SARS-CoV-2 infection and were suspected of having CAPA.

4.2. Demographic and clinical data

Demographic and clinical data were retrospectively extracted from electronic medical records. The demographic data included age and gender, clinical data included wards of stay, date of admission and discharge, host risk factors for immunosuppressive condition according to aspergillosis diagnostic criteria, other relevant comorbidities, use of invasive or non-invasive mechanical ventilation, clinical outcome. Microbiological data included beta-D-glucan, fungal cultures and galattomannan (GM) antigen results from any sample. Chest X-rays or computed tomography (CT) results were reported as "normal" or with "typical" and "atypical" alteration. Antifungal therapy choice was also collected. Ninety-one patients had at least one missing value.

4.3. Laboratory and radiological investigations

Regarding laboratory investigations, we considered positive any galattomannan ≥0.5, regardless of biological sample, as standard practice in our microbiology laboratory. We included also 1,3-Beta-D-glucan turbidimetric assay using as cutoff 7 pg/ml. Culture testing for *Aspergillus* spp. was performed in all patients in whom representative material from the lower respiratory tract could be collected.

Typical radiological patterns, evidenced on X-Ray or CT-scan examinations, include the following: dense, well-circumscribed lesion(s) with or without halo sign; air-crescent sign; a cavity; segmental or lobar consolidation. These typical signs are uncommon in non-neutropenic patients.

Atypical radiological patterns refer to presence of elements like unspecific infiltrates, diffuse ground-glass opacity and consolidations.

4.4. Cases' definitions according to criteria

Patients were classified as cases of CAPA according to EORTC/MSG, modified AspICU and ECMM criteria with a few modifications:

- EORTC/MSG host factors related to an immunosuppressive condition [7] were applied as standard, but we equated prolonged use of dexamethasone or methylprednisolone prescribed for SARS-CoV-2 pneumonia to prolonged, high-dose of prednisone (≥ 0.3 mg/kg for more than 3 weeks).
- Among the AspICU criteria we chose the modified AspICU [10], applied as standard. However, the entry criterion "ICU admission" was considered present also in patients receiving mechanical ventilation in ordinary wards, since these patients would have been admitted to the ICU prior to the pandemic.
- ECMM criteria were applied faithfully [12].

4.5. Statistical analysis

The patients' clinical numerical characteristics were presented as median and interquartile ranges after the Shapiro test excluded the normal hypothesis, while categorical variables were presented with frequencies and percentages.

The concordance analyses between the three criteria and the DTT, (defined as the physician's decision to assign an anti-fungal treatment to the patient) and the agreement between the criteria themselves required the first step of making the criteria binary. Patients who could not be assigned to a diagnostic category within a given criterion (for example for lack of the entry factor) or who were assigned to the "colonisation" category were given value 0; while patients assigned to the possible, probable or putative categories were given value 1. We then built 2x2 tables with the binary categories and the DTT. From these tables we calculated Cohen's Kappa coefficient and its p-value with the z-test.

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We then evaluated through a univariate analysis the differences between the variables used to compile the diagnostic criteria, after dividing the study cohort according to the DTT. We used the non-parametric Mann-Whitney test to compare the numerical variables and the Chi-square or Fisher's exact test, when appropriate, to compare the categorical variables binarised in 0/1 values. The same univariate analysis was repeated for the outcome of "survival".

Finally, we assessed with a multivariate analysis the possible association between survival and use of AspICU, ECMM and EORTC/MSG criteria. We used logistic regression and included the criteria as predictors and the DTT variable as an additional confounding factor. Results of the multivariable analysis are reported as odds ratio (OR) and 95% confidence interval.

All the statistical tests were conducted two-tails and a p-value<0.05 was considered significant. Rstudio 4.0.5. with R version 4.1.2 was used for all the computation and statistical analysis.

4.6. Informed consent and ethical concerns

All patients provided informed consent for the use of clinical data for scientific purpose according to hospital policy. Administrative data about our hospital's COVID-19 patients are collected in a registry (SMACORE), approved by the Fondazione IRCCS Policlinico San Matteo's ethics committee with protocol number 20200046877.

5. Conclusions

The evidence gathered in this study suggests that the use of EORTC/MSGERC criteria to guide the diagnosis is not considered applicable in clinical practice by physician due to lack of inclusion of clinical signs and symptoms and does not lead to improved survival. AspICU and ECMM criteria, on the other hand, showed a good degree of agreement with the DTT and between themselves and a positive correlation with patient recovery.

Author Contributions: Conceptualization, E.A.; methodology, G.A. and P.S.; software, G.A.; validation, C.C. P.S., R.B.; formal analysis, G.A.; investigation, F.C., R.P, C.B., M.C., C.C.; resources, R.B.; data curation, F.C., R.P, C.B. M.C., S.L.; writing—original draft preparation, E.A, F.C., R.P, C.B. M.C.; writing—review and editing, P.S., C.C., R.B.; visualization, G.A.; supervision, E.A., P.S.; project administration, R.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and collection of data under the SMACORE database was approved by the Institutional Review Board (or Ethics Committee) of Fondazione IRCCS Policlinico San Matteo (protocol code 20200046877).

Informed Consent Statement: All patients provided informed consent for the use of clinical data for scientific purpose according to hospital policy.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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