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ORIGINAL RESEARCH—CLINICAL

Circulating IgG4$^+$ Plasmablast Count as a Diagnostic Tool in Autoimmune Pancreatitis

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BACKGROUND & AIMS: Type 1 autoimmune pancreatitis (AIP) is an IgG4-related disease whose diagnosis is challenging. The aim of this study was to investigate the diagnostic value of circulating total and IgG4$^+$ plasmablasts in differentiating this condition from the other main pancreatic diseases. **METHODS:** Patients with type 1 AIP (n = 19) were prospectively enrolled in a tertiary center together with patients suffering from type 2 or not otherwise specified (NOS) AIP (n = 10), pancreatic adenocarcinoma (n = 17), chronic pancreatitis (n = 20), and intraductal papillary mucinous neoplasia or chronic asymptomatic pancreatic hyperenzymemia (n = 21) as control groups. Flow cytometry was used to measure the total plasmablast and IgG4$^+$ plasmablast number by gating peripheral blood CD45$^+$CD19$^+$CD38^{hi}CD20$^-$CD24$^-$CD27$^+$ and CD45$^+$CD19$^+$CD38^{hi}CD20$^-$CD24$^-$CD27$^+$IgG4$^+$ cells, respectively. In patients with AIP, these cell populations were also evaluated after 1 month of therapy, after 2–4 months from the end of treatment, and after 1 year from the enrollment. The study was approved by the local ethics committee (protocol number: 59133, 30/11/2017). **RESULTS:** Total plasmablast quantification was capable of discriminating type 1 AIP from all the other pancreatic disorders with a sensitivity of 47% and a specificity of 81%, according to a cutoff of 4500 cells/mL (AUC = 0.738), whereas IgG4$^+$ plasmablast count distinguished type 1 AIP from all the other pancreatic disorders with a sensitivity of 80% and a specificity of 97% when applying a cutoff of 210 IgG4$^+$ cells/mL (AUC = 0.879). The basal IgG4$^+$ plasmablast number was significantly higher (P = .0001) in type 1 AIP than in type 2/NOS AIP, decreased after steroid therapy, and increased at disease relapse. **CONCLUSION:** IgG4$^+$ plasmablast count represents a potentially useful biomarker to differentiate type 1 from type 2/NOS AIP and from other pancreatic diseases.

Keywords: Autoimmune Pancreatitis; Biomarker; Diagnosis; Plasmablasts

Introduction

Autoimmune pancreatitis (AIP) is a rare chronic inflammatory disease involving part or the whole organ that dramatically responds to systemic steroids and may relapse unpredictably.[1] Two forms can be identified according to the histological features: type 1 or lymphoplasmacytic sclerosing pancreatitis and type 2 or idiopathic duct–centric pancreatitis.[2] Despite both presenting with similar clinical and imaging features, only the former is considered an IgG4-related disease (IgG4-RD), characterized by elevated IgG4 serum levels and abundant IgG4$^+$ plasma cell infiltration of the target organ.[3] However, since the histologic examination is not always feasible or diagnostic, the differentiation between the 2 forms is usually achieved by applying the International Consensus Diagnostic Criteria.[4] When a definitive or probable diagnosis of type 1 or 2 cannot be achieved, AIP is defined as not otherwise specified (NOS).[4] A further and common clinical challenge is the need to differentiate AIP from pancreatic cancer because of similar clinical and radiological features. So far, the only available biomarker is the dosage of IgG4 serum levels that has 65% sensitivity and 98% specificity in diagnosing type 1 AIP for levels higher than 135 mg/dL.[5] However, its diagnostic value is limited because it cannot be used for type 2 and NOS forms, and it is also increased in a proportion of patients with pancreatic adenocarcinoma (PDAC).[6,7] In this scenario, the availability of a biomarker useful to differentiate type 1 AIP from the other main pancreatic diseases is awaited, also considering that

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Abbreviations used in this paper: AIP, autoimmune pancreatitis; BMI, body mass index; CAPH, chronic pancreatic hyperenzymemia; CP, chronic pancreatitis; IgG4-RD, IgG4-Related Disease; IPMN, intraductal papillary mucinous neoplasia; IQR, interquartile range; NOS, not otherwise specified; PDAC, pancreatic adenocarcinoma; ROC, receiver operating characteristic; SD, standard deviation.

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Table. Demographic and Clinical Features of the Study Population

Features	AIP-1 group	AIP-2/NOS group	PDAC group	CP group	IPMN-CAPH group
Number of cases	19	6/4	17	20	21
Mean age in y (SD)	61 (14)	43 (18)	66 (9.1)	43 (15.3)	43 (18)
Male/female ratio (male %)	18:1 (95)	6:4 (60)	13:4 (76.5)	15:5 (75)	12:9 (57)
Mean BMI (SD)	26 (10)	23 (3.9)	21 (3.1)	22 (2.9)	22 (2.9)
Number of active smokers (%)	6 (31)	4 (40)	10 (59)	13 (65)	8 (38)
Number of active drinkers (%)	3 (16)	0 (0)	4 (23)	7 (35)	5 (23)
Median disease duration in mo (range)	23.8 (2–192)	4 (2–25)	4 (2–36)	36 (3–216)	24 (6–180)

AIP, autoimmune pancreatitis; BMI, body mass index; CAPH, chronic asymptomatic pancreatic hyperenzymemia; CP, chronic pancreatitis; IPMN, intraductal papillary mucinous neoplasia; NOS, not otherwise specified; PDAC, pancreatic adenocarcinoma; SD, standard deviation.

misdiagnosis between PDAC and AIP may lead to unnecessary surgery in up to 27% of cases.[8]

Plasmablasts, a cell population belonging to the B-cell lineage, are an intermediate stage between activated B-cells and plasma cells.[9] Their peripheral blood rate is generally very low in healthy people,[10,11] while increasing transiently during infections or vaccination.[12] Instead, long-lasting elevation occurs in autoimmune diseases, such as inflammatory bowel diseases, rheumatoid arthritis, systemic lupus erythematosus.[10,11,13–16] In IgG4-RD, treatment with steroids or rituximab (a monoclonal antibody targeting the B-cell marker CD20) determines the almost-complete clearance of circulating plasmablasts that may re-emerge after drug withdrawal.[17–19] In addition, the $IgG4^+$ plasmablast number seems to correlate with disease activity in the IgG4-RD context,[9,17,20] although its diagnostic and prognostic value in AIP has never been assessed.

The aim of this study was to assess the levels of circulating plasmablasts, both as the total and as $IgG4^+$ cell number, in AIP in comparison with the main pancreatic disorders, to investigate their utility as a diagnostic and prognostic biomarker.

Patients and Methods

Study Population

A total of 87 adult patients were prospectively enrolled at the Gastroenterology Unit, Pancreas Institute, A.O.U.I. Policlinico G.B. Rossi & University of Verona (Verona, Italy) from January 1st, 2018, through May 30th, 2020. They included 19 patients with type 1 AIP (AIP-1 group) as index population and 10 patients with type 2 or NOS AIP (AIP-2/NOS group), 17 patients with PDAC (PDAC group), 20 patients with chronic pancreatitis (CP) (CP group), and 21 subjects with intraductal papillary mucinous neoplasia (IPMN) or chronic asymptomatic pancreatic hyperenzymemia (CAPH) (IPMN-CAPH group) as control groups. The demographic and clinical features of the enrolled cases are shown in Table where, as expected, an older age and a prevalence of male gender are evident in AIP-1 and PDAC groups, while a prevalence of active smokers appears in PDAC and CP groups. The diagnosis of AIP was based on the International Consensus Diagnostic Criteria.[4] Only patients naïve to or free from any immunosuppressive treatment (steroids, biologics, or immunosuppressive drugs) in the last 6 months were enrolled. All patients with AIP underwent treatment with prednisone at a daily dosage of 0.6–1 mg/kg body weight p.o. for 4 weeks, tapered by 5 mg/wk until discontinuation, and abdominal magnetic resonance imaging (MRI) at baseline (timepoint 0), after 1 month of therapy (timepoint 1), after 2–4 months from the end of treatment (timepoint 2), and after 1 year from the enrollment (timepoint 3). Two expert clinicians (LF and AA) re-evaluated all MRI examinations at these timepoints to assess whether the disease was stable, in remission or in relapse. In addition, disease activity in the AIP-1 group was assessed at each timepoint by applying the IgG4-RD Responder Index (IgG4-RD RI), including patient's clinical assessment, IgG4 serum levels, organ involvement and damage, and need for an urgent treatment.[18] Patients with disease relapse requiring rituximab or long-term steroids were censored at timepoints 1 and 2, respectively. Moreover, patients at the second relapse, who underwent maintenance treatment with low-dose steroids, were censored at timepoint 2. Only patients with a biopsy-proven diagnosis were enrolled in the PDAC group, while the CP group included those diagnosed according to the United European Gastroenterology evidence-based guidelines.[19] Finally, patients with diagnosis of IPMN of the main pancreatic duct and/or branch ducts, as assessed by MRI cholangiopancreatography, were enrolled in the IPMN-CAPH group together with subjects with CAPH, defined as a condition characterized by persistent high levels of serum amylase and lipase for more than 6 months in the absence of pancreatic disease.[21]

Patients with AIP underwent peripheral blood sample collection at each timepoint to quantify both total and $IgG4^+$ plasmablasts (see Supplemental Material) as well as IgG4 serum levels. Control groups were tested only once at timepoint 0. Patients were excluded in case of blood or bone marrow donation/transfusion within 2 months of the screening, history of hematological disorders, bone marrow transplantation, pancreatic surgery, or immunosuppressive therapy within 6 months before enrollment. The study was approved by the local ethics committee (protocol number 59133, 30/11/2017), and each enrolled case provided written informed consent to participate.

Statistical Analysis

Baseline demographic and disease findings are presented by using descriptive statistics. Continuous variables are described as mean ± standard deviation (SD) or median ± interquartile range (IQR). Both total and IgG4$^+$ plasmablast rates were compared among groups using the Mann-Whitney test or Wilcoxon test, as appropriate. Both total and IgG4$^+$ plasmablast counts were correlated with clinical parameters by applying the Spearman's rank-order or Pearson product-moment correlation when discrete or continuous variables were involved, respectively. The receiver operating characteristic (ROC) analysis was used to identify the plasmablast cutoff value for discrimination between type 1 AIP and the other control groups. A value of $P < .05$ was considered statistically significant. Statistical analyses were performed using Prism software, version 8.0 (GraphPad Software, La Jolla, CA).

Results

Clinical Findings of the Index Population

All patients enrolled in the AIP-1 group displayed elevated IgG4 serum levels at timepoint 0 [mean: 624.2 (SD: 485) mg/dL; normal values < 135], which decreased over time (timepoint 1: 277.2 [SD: 208]; timepoint 2: 379 [SD: 295] with 1 patient excluded because of relapse; timepoint 3: 297 [SD: 198], with 3 cases excluded because of relapse). At variance with patients with AIP-1, those included in the AIP-2/NOS group showed levels of IgG4 lower than the cutoff value (mean: 59.7, SD: 45 mg/dL; normal values < 135). The pancreas was the only organ affected in 9 patients (47%), whereas a combination of multiorgan involvement was evident in 10 cases (53%): biliary tree in 9, kidneys in 4, lymph nodes in 2, salivary glands in 1, and aorta in 1. Moreover, 13 patients (68%) presented with a diffuse organ disease, whereas 6 patients (32%) showed a focal pancreatic disease. Among these, only one displayed a Ca-19.9 level above the normal limit (74 KU/L, normal value < 39 KU/L), while 2 of them were "not expressors" (values < 9 KU/L), and 3 cases had obstructive jaundice that affects the diagnostic value of this marker, so we did not carry out the measurement. Nine cases (47%) underwent endoscopic ultrasound examination with fine-needle aspiration whose cytology examination showed type 1 AIP in 5 cases, no signs of cancer in 3 cases, and very likely cancer in 1 case who received a final diagnosis of type 1 AIP upon pancreaticoduodenectomy. Concerning the therapeutic status at enrolment, 14 patients (73%) were naïve to treatment, 3 were at their first relapse after a previous course of steroid therapy, one was enrolled during a relapse occurring 1 year after the end of treatment with azathioprine (2.0 mg/kg body weight), and one during a relapse 2 years from the end of rituximab (1000 mg i.v. at day 0 and 15, repeated after 6 months). As regards disease activity, the median value of the IgG4-RD RI at baseline was 10 (IQR: 6), dropping to 2 (IQR: 1), 3 (IQR: 4), and 2 (IQR: 2) at timepoints 1, 2, and 3, respectively. Finally, 11 of 19 patients did not complete the follow-up: 3 dropped out at timepoint 1 (2 underwent rituximab treatment and one refused steroid therapy) and 8 at timepoints 2 or 3 (6 lost to the follow-up, one underwent long-term steroid therapy, and one relapsed and started a further cycle of steroids).

Total Plasmablast Count at Baseline

As shown in Figure 1A, the mean level of circulating total plasmablasts at baseline was higher in the AIP-1 group than in the PDAC group (mean: 6365, SD: 5522 cells/mL vs mean: 3216, SD: 1228 cells/mL; $P = .0067$), despite the presence of an outlier in the PDAC group (21,900 cells/mL). If dissecting the data of the AIP-1 group among those with focal (mean: 6992, SD: 7569 cells/mL) and diffuse (mean: 6077, SD: 4650 cells/mL) pancreatic involvement, the difference was statistically significant only when comparing the latter subgroup with patients with PDAC ($P = .006$). Even the IPMN-CAPH group displayed a mean rate of circulating plasmablasts lower than the AIP-1 group (mean: 1065, SD: 781 cells/mL; $P < .0001$), whereas no statistically significant difference was found between the AIP-1 group and AIP-2/NOS group (mean: 3318, SD: 3025 cells/mL; $P = .075$), possibly due to the presence of a significant outlier in the latter group (11,410 cells/mL). No statistical difference was found between the AIP-1 group and CP group (mean: 4084, SD: 2272 cells/mL; $P = .177$). Notably, the IPMN-CAPH group showed the lowest plasmablast frequency, even lower than both PDAC and CP groups ($P = .0082$ and .0001, respectively). Finally, the plasmablast rate at baseline in the AIP-1 group showed a strong positive correlation with the IgG4-RD RI (rho = 0.6, $P = .007$; Figure 1B), the grade of multiorgan involvement (rho = 0.7, $P = .025$; Figure 1C), and IgG4 serum levels (rho = 0.6, $P = .007$; Figure 1D). Finally, the ROC curve analysis showed that a cutoff of 4500 cells/mL had a sensitivity of 47% and specificity of 81% for differentiating type 1 AIP from all the other groups (area under the curve = 0.738) (Figure 2).

IgG4$^+$ Plasmablast Count at Baseline

When selecting the plasmablasts according to the expression of IgG4, the number of circulating IgG4$^+$ cells resulted significantly higher in the AIP-1 group (mean 1177, SD 1712 cells/mL) than in the AIP-2/NOS group (mean: 35, SD: 73 cells/mL; $P = .0001$; Figure 3A). Similarly, those 6 patients with focal type 1 AIP displayed a high mean value (mean: 2453 cells/mL). However, 3 of 19 patients with type 1 AIP did not show detectable levels of IgG4$^+$ plasmablasts, while 2 of 10 patients with type 2/NOS AIP displayed circulating IgG4$^+$ plasmablasts, although with values consistently lower (200 and 119 cells/mL, respectively) than those observed in patients with type 1 AIP. Moreover, no correlation was found among the IgG4$^+$ plasmablast count and the IgG4-RD RI (rho = 0.1, $P = .72$; Figure 3B), multiorgan involvement (rho = −0.13, $P = .77$; Figure 3C), and IgG4 serum levels (rho = 0.47, $P = .076$; Figure 3D) in the AIP-1 group. Finally, 1 patient with CP showed circulating IgG4$^+$ plasmablasts (732 cells/mL), while no case suffering from PDAC, IPMN, and CAPH displayed detectable IgG4$^+$ plasmablasts. At the ROC curve analysis, a cutoff of 210 IgG4$^+$

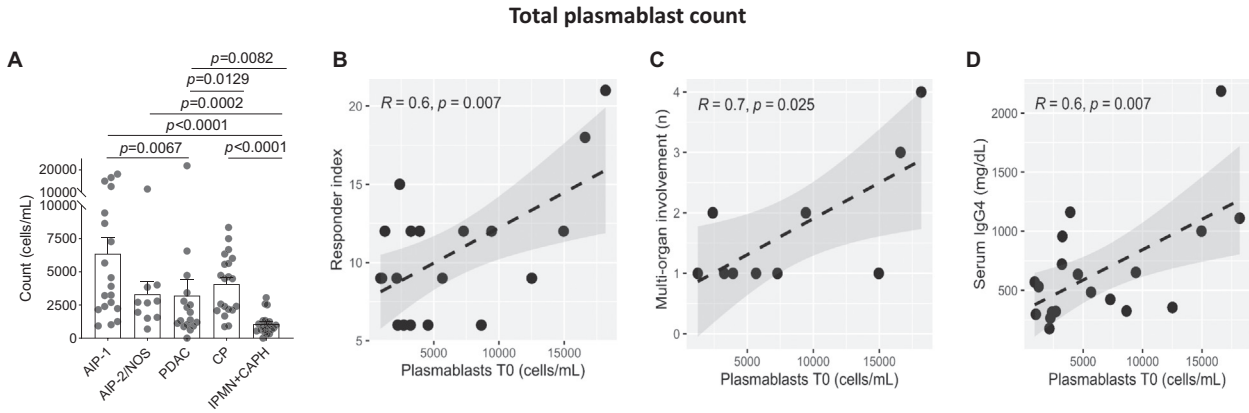

Figure 1. Total plasmablast counts. Comparison of the total plasmablast counts, expressed as cells/mL, detected in AIP-1 (n = 19), AIP-2/NOS (n = 10), PDAC (n = 17), CP (n = 20), and IPMN-CAPH (n = 21) groups at baseline (A). The statistically significant differences among groups are shown. Correlation among the total plasmablast number and IgG4-related disease responder index (B), multiorgan involvement (C), and IgG4 serum levels (D) in the AIP-1 group at baseline. For comments see the text. Ig, immunoglobulin.

plasmablasts/mL had a sensitivity of 80% and specificity of 97% for differentiating type 1 AIP from all the other groups (area under the curve = 0.879) (Figure 4).

Total and IgG4$^+$ Plasmablast Counts Over Time

As shown in Figure 5, during the follow-up period, we observed a progressive decrease of circulating total plasmablasts in patients with AIP-1 at timepoint 1 (mean: 5124, SD: 5790 cells/mL), achieving the statistical significance at timepoint 2 (mean: 3259, SD: 3520 cells/mL; $P = .0239$) and even more at timepoint 3 (mean: 1856, SD: 1751 cells/mL; $P = .0070$). At variance, the mean of the IgG4$^+$ cell subset dramatically decreased at timepoint 1 (mean: 224, SD: 321 cells/mL; $P = .0149$) and remained substantially unchanged over time (mean: 314, SD: 429 cells/mL; $P = .600$ at timepoint 2 and mean: 115, SD: 191 cells/mL; $P = .0157$ at timepoint 3). Remarkably, 3 patients experiencing disease flare (1 at timepoint 2, 2 at timepoint 3) displayed high values of total plasmablasts (ie, 4878, 4868, and 1148 cells/mL), IgG4$^+$ plasmablasts (ie, 652, 295, and 220 cells/mL), and IgG4 serum levels (ie, 840, 227, and 167 mg/dL) at relapse time. By contrast, no significant changes were observed in the patients with AIP-2/NOS as far as total plasmablasts (mean: 7492, SD: 7000 cells/mL at timepoint 1; mean: 3230, SD: 3203 cells/mL at timepoint 2; mean: 8111, SD: 13,239 cells/mL at timepoint 3) and IgG4$^+$ plasmablasts (not detected at timepoints 2 and 3; mean: 56 cells/mL, range: 0–282 cells/mL, SD 126 cells/mL at timepoint), despite treatment was established in all cases.

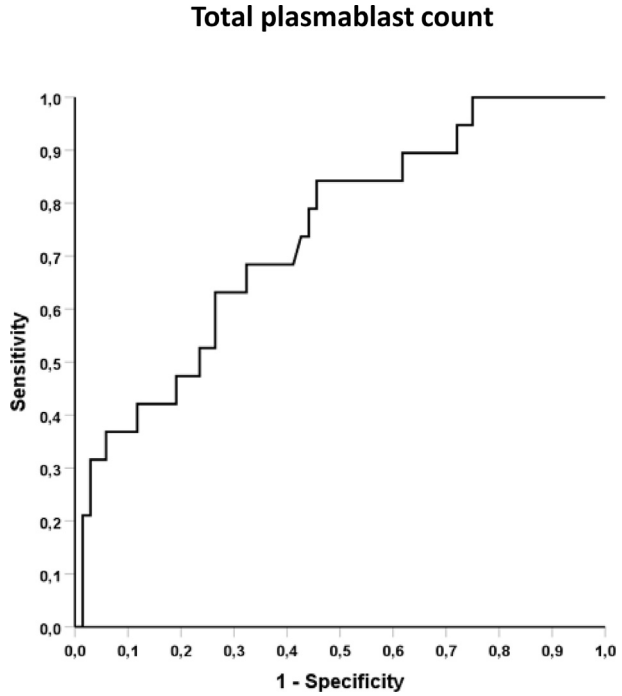

Figure 2. ROC curve analysis of total plasmablast counts. The graphical plot of the receiver operating characteristic (ROC) analysis showed that a threshold of 4500 cell/mL discriminated type 1 AIP from the other groups with a sensitivity of 47% and a specificity of 81%.

Discussion

AIP represents a clinical challenge due to both diagnostic difficulty and high relapse rate, particularly in type 1. Indeed, the diagnostic algorithm includes invasive procedures, such as percutaneously or endoscopic ultrasound-guided fine-needle aspiration and, in a limited number of cases, the final diagnosis is eventually reached only through surgery. In this clinical context, the availability of a noninvasive biomarker, with a high predictive value for differentiating AIP from PDAC, is still an unmet need. In addition, disease relapse, frequent during the first 2 years from the onset, cannot be predicted.[22] Recent studies reported the clinical usefulness of circulating plasmablast enumeration in the setting of IgG4-RD. In particular, Wallace et al[9] showed

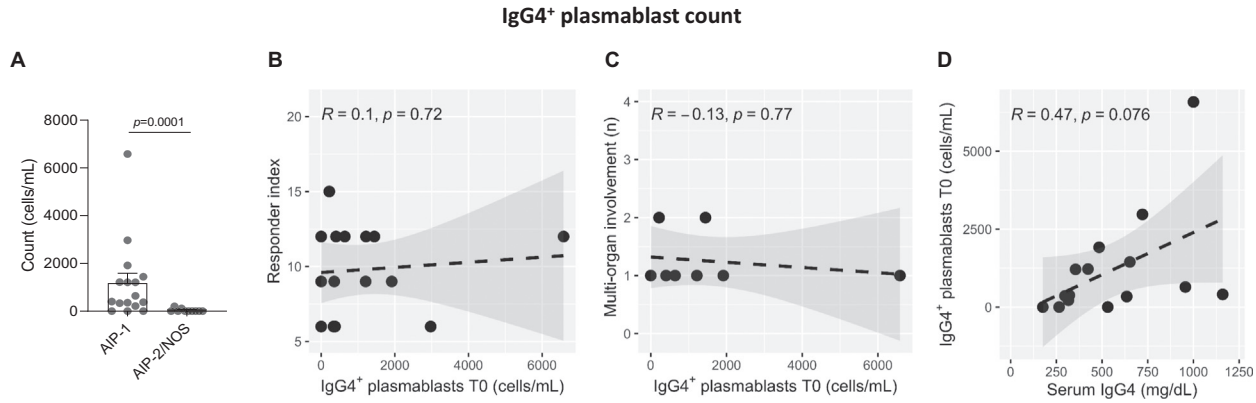

Figure 3. IgG4$^+$ plasmablast counts. Comparison of the IgG4$^+$ plasmablasts counts, expressed as cells/mL, detected in AIP-1 (n = 16) and in AIP-2/NOS (n = 9) groups at baseline (A). A statistically significant higher mean value was found in the AIP-1 group with respect to the AIP-2/NOS group. Correlation among IgG4$^+$ plasmablast levels and the IgG4-related Disease Responder Index (B), multiorgan involvement (C), and IgG4 serum levels (D) in the AIP-1 group at baseline. For comments see the text. Ig, immunoglobulin.

that high levels of both total plasmablasts (median value: 4698/mL) and IgG4$^+$ plasmablasts (median value: 2808/mL) can be detected in the peripheral blood of patients suffering from active and treatment-naïve IgG4-RD, as compared to both healthy subjects and patients with other autoimmune diseases; in addition, a total plasmablast count >2000/mL was suggested as a highly specific marker of IgG4-RD. Remarkably, both values underwent significant decrease following a cycle of treatment with rituximab-mediated B-cell depletion. However, in this cohort, pancreatic involvement was present only in 8 of 37 cases. Similarly, Mattoo et al[19] demonstrated the presence of significantly higher levels of circulating CD19$^+$CD27$^+$CD38^{hi} plasmablasts, decreasing upon rituximab, in 84 patients with IgG4-RD than in 16 healthy controls. Furthermore, Akiyama et al[23] reported a high mean number of total plasmablasts (3294 cell/mL, SD: 1483 cell/mL) in a series of 15 active, untreated patients with IgG4-RD (only one with pancreatic involvement), again significantly decreasing following steroid therapy. These data were subsequently confirmed, including the ability of steroids to lower plasmablast levels.[24] Following this evidence, we aimed at exploring whether this analysis was suitable in the specific setting of AIP and specifically predictive for this disease as compared to the other main pancreatic disorders. Our results show that the total plasmablast count may help to distinguish type 1 AIP from other conditions, but with an unsatisfactory sensitivity (47%) and specificity (81%) using the best cutoff value (4500 cells/mL) in the ROC curve. Even if applying the Wallace cutoff of 2000 cells/mL (9), sensitivity was increased (84%), but with a very low specificity (51%). Furthermore, no difference was found when comparing the total plasmablast count of the PDAC group with focal type 1 AIP, possibly due to the tiny number of cases of this subgroup (n = 6). However, it is worth noting the robust positive correlation between total plasmablasts at baseline in AIP-1 group and all parameters routinely evaluated, such as IgG4 serum levels, multiorgan involvement, and IgG4-RD RI. This is a remarkable point, as Wallace et al[9] did not find any correlation between the median values of peripheral plasmablasts and IgG4 serum levels in active IgG4-RD. Furthermore, it should be emphasized that among patients with focal type 1 AIP, only one of the 3 tested showed detectable levels of serum CA-19.9 (χ^2 upper normal value), while in the PDAC group, all but 3 cases displayed high levels (mean value: 282 KU/L, SD: 309; normal value < 39

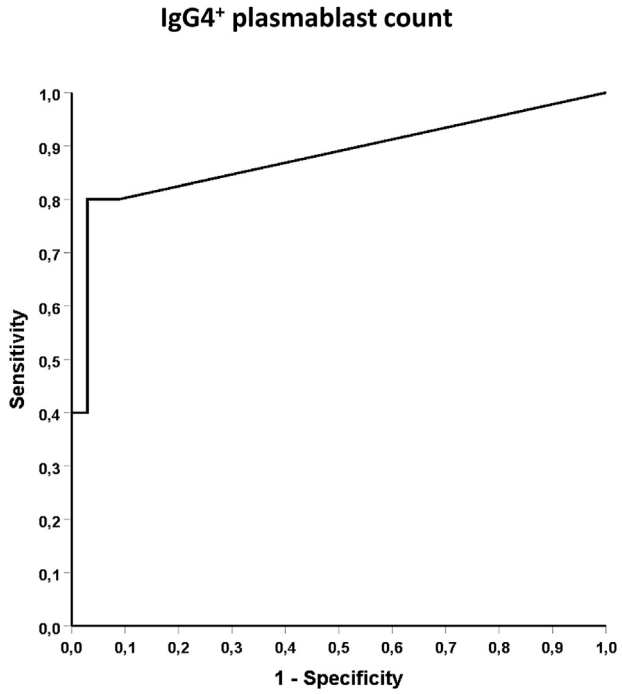

Figure 4. ROC curve analysis of IgG4$^+$ plasmablast counts. The graphical plot of the receiver operating characteristic (ROC) analysis showed that a threshold of 210 cell/mL discriminated type 1 AIP from the other groups with a sensitivity of 80% and a specificity of 97%.

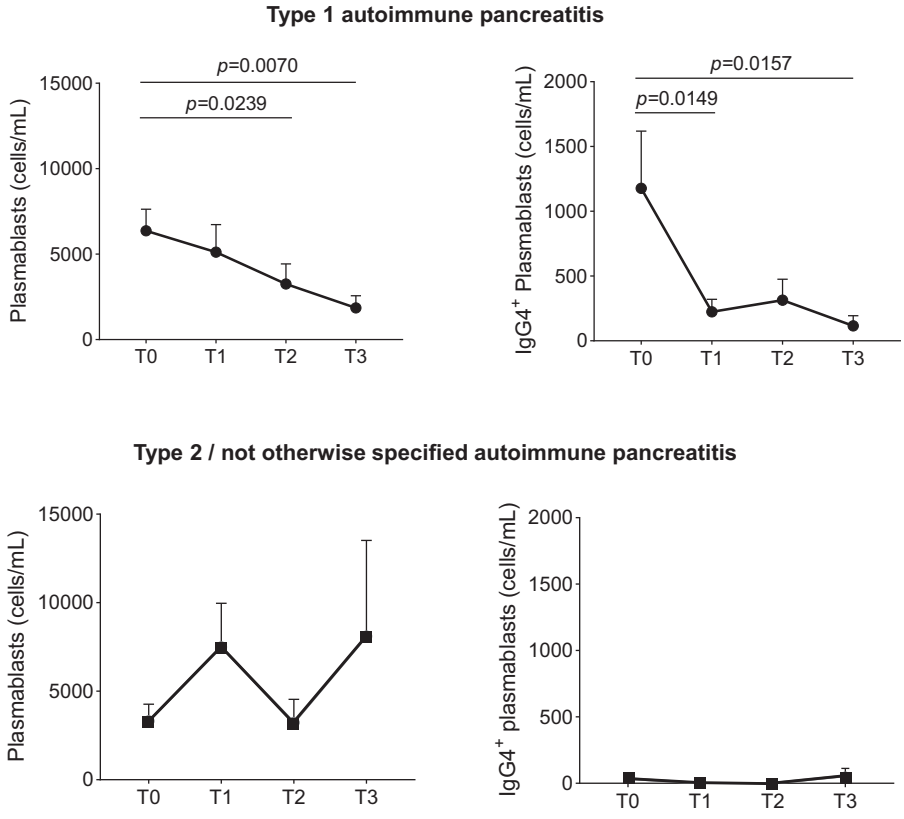

Figure 5. Total and IgG4$^+$ plasmablast counts in autoimmune pancreatitis patients over time. Total plasmablast (upper left-side panel) and IgG4$^+$ plasmablast (upper right-side panel) counts detected in type 1 autoimmune pancreatitis at T0 (total n = 19, IgG4$^+$ n = 16), T1 (total n = 13, IgG4$^+$ n = 11), T2 (total n = 9, IgG4$^+$ n = 7), and T3 (total n = 6, IgG4$^+$ n = 6). A critical decrease of IgG4$^+$ plasmablast count appears evident as soon as treatment was undertaken, whereas a slow decrease of the total plasmablast count was shown over time. Total plasmablast (lower left-side panel) and IgG4$^+$ plasmablast (lower right-side panel) counts detected in type 2/not otherwise specified autoimmune pancreatitis at T0 (total n = 10, IgG4$^+$ n = 9), T1 (total n = 8, IgG4$^+$ n = 7), T2 (total n = 6, IgG4$^+$ n = 4), and T3 (total n = 6, IgG4$^+$ n = 5). No significant changes were observed in both cases, despite treatment was established in all cases. Ig, immunoglobulin; T, timepoint.

KU/L), so the final diagnosis was established only upon biopsy.

Searching for more valuable diagnostic tools and biomarker predictors of disease course, we implemented the immunophenotypic characterization of circulating plasmablasts with the class of immunoglobulin representing a hallmark of type 1 AIP. Indeed, a cutoff of 210 IgG4$^+$ plasmablasts/mL seems to distinguish type 1 AIP from all the other pancreatic disorders, with remarkable values of sensitivity (80%) and specificity (97%). Indeed, in almost all patients with PDAC, CP, IPMN, and CAPH, no detectable values of this cell subset were found, whereas a mild increase (<210 mg/mL) was observed in 2 cases with type 2/NOS AIP at timepoint 0 and 1 patient with CP with multiple pseudocysts and diffuse inflammatory thickening of the gastric and duodenal wall (732 cells/mL). Notably, 3 type 1 AIP cases, all displaying serum IgG4, did not show detectable IgG4$^+$ plasmablasts, a finding that deserves wider investigation. Finally, the rate of circulating IgG4$^+$ plasmablasts in AIP underwent dramatic reduction as soon as steroid therapy was undertaken, while the total plasmablast count decreased more slowly. Thus, circulating IgG4$^+$ plasmablast enumeration seems to be a more sensitive marker of disease activity in AIP than the total plasmablast count. This feature seems to be disease-specific, as in the AIP-2/NOS group no substantial modification of the total plasmablasts frequency was observed over time, and the IgG4$^+$ subset was persistently negligible.

Regarding the 3 AIP-1 cases who relapsed during the follow-up, our analysis did not demonstrate any clinical usefulness of total plasmablast enumeration, as the values were quite heterogeneous. In contrast, all relapsing patients had values of circulating IgG4$^+$ plasmablasts over the chosen cutoff (210 cells/mL). This result supports the potential predictive value of IgG4$^+$ plasmablast quantification when suspecting a disease recurrence.

The main limitation of this kind of studies is that AIP is a rare condition so that the sample size is inevitably small. Nevertheless, our prospective and monocentric study included all the main pancreatic disorders as control groups, and this contributes to the robustness of the results. Furthermore, the clear definition and classification of the study groups, the fixed time points for disease evaluation, and the standardized multiparametric flow cytometry–based approach to enumerating circulating IgG4$^+$ plasmablasts allowed us to achieve reliable results that deserve to be confirmed through further prospective and multicenter studies in larger cohorts of patients.

In conclusion, our data suggest that circulating IgG4$^+$ plasmablasts may represent an important tool for diagnosis of type 1 AIP and its differentiation from type 2 AIP/NOS and PDAC. In addition, IgG4$^+$ plasmablast quantification seems helpful for monitoring response to therapy and disease relapse in type 1 AIP. Thus, this noninvasive, highly

reproducible and cost-affordable analysis may represent a suitable biomarker for application in clinical practice in the suspicion and management of type 1 AIP.

Supplementary Materials

Material associated with this article can be found in the online version at https://doi.org/10.1016/j.gastha.2022.02.012.

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Authors' Contributions:
Rachele Ciccocioppo and Giulia De Marchi designed the study, analyzed and interpreted the data, and wrote the manuscript. They have approved the final draft submitted. Valeria Zuliani and Annalisa Adamo carried out the flow cytometric analyses and performed the statistical analysis. They have approved the final draft submitted. Antonio Amodio and Pietro Campagnola were in charge of the management of the patients. They have approved the final draft submitted. Enrico Maria Gabrieletto and Nicolò de Pretis were involved in collecting patients' clinical data and technical support. They have approved the final draft submitted. Stefano Ugel and Pietro Delfino collected the bibliography and helped in drafting and formatting the manuscript. They have approved the final draft submitted. Mauro Krampera critically revised the

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The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Data Transparency Statement:
The data, analytic methods, and study materials will be made available to other researchers upon reasonable request and sent as document files.