



Correlation Between iXip and Final Pathology in Patients Affected by Prostate Cancer Undergoing Radical Prostatectomy: A Multicenter Prospective Trial (PROXIMA—PROstate iXip Index Multicenter Analysis)

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*Correspondence:

Simone Francavilla
simone.francavilla@poliambulanza.it

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Simone Francavilla^{1,2,3*}, Chiara Lonati^{1,2}, Marco Sandri⁴, Andrea Abate⁵,
Enrico De Marzo^{1,2}, Carlotta Palumbo^{1,2,6}, Lucia Aretano^{1,2}, Stefano Belotti^{1,2},
Angelo Peroni^{2,3}, Alessandro Peviani⁷, Stefania Ferretti⁷, Luigi Filippo Da Pozzo⁸,
Marco Roscigno⁸, Stefano Calza⁵, Agostino Guerini⁹, Sandra Sigala⁵, Claudio Simeone^{1,2}
and Alessandro Antonelli^{10,11}

¹ Urology Unit, Azienda Socio Sanitaria Territoriale (ASST) Spedali Civili Hospital, Brescia, Italy, ² Department of Medical and Surgical Specialties, Radiological Science, and Public Health, University of Brescia, Brescia, Italy, ³ Urology Unit, Department of Surgery, Istituto Fondazione Poliambulanza, Brescia, Italy, ⁴ Big & Open Data Innovation Laboratory (BODaL-Lab), University of Brescia, Brescia, Italy, ⁵ Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy, ⁶ Department of Urology, University of Eastern Piedmont, Maggiore della Carità Hospital, Novara, Italy, ⁷ Department of Urology, University Hospital of Parma, Parma, Italy, ⁸ Department of Urology, Azienda Socio Sanitaria Territoriale (ASST) Papa Giovanni XXIII of Bergamo, Bergamo, Italy, ⁹ Department of Molecular and Translational Medicine, Pathology Unit, Azienda Socio Sanitaria Territoriale (ASST) Spedali Civili of Brescia, Brescia, Italy, ¹⁰ Urology Unit, Azienda Ospedaliero Universitaria Integrata di Verona, Verona, Italy, ¹¹ Department of Surgery, Dentistry, Pediatrics and Gynecology, University of Verona, Verona, Italy

Introduction: The Immune complex Predictive Index (iXip) is a predictive tool for prostate cancer (PCa) diagnosis that integrates PSA, PSA-IgM, prostate volume, and patient age. The aim of the study was to assess the correlation between iXip and clinically significant PCa in patients who underwent radical prostatectomy.

Material and Methods: A prospective multicenter study was conducted from February 2018 to August 2019 enrolling 235 patients. Stepwise-selected predictors were used to estimate multivariate regression models for each outcome, the reference model with only the set of predictors and the same model with the addition of iXip. The prediction accuracy of the two models was assessed calculating the partial area under the receiver operating characteristic curve.

Results: The ROC curve analysis showed significant differences in terms of partial area under the curve between iXip and pathological Gleason Score ≥ 7 and between iXip and tumor volume ≥ 2.5 mm³. The scatter plot analysis showed a positive linear correlation between iXip and tumor volume (considered as a continuous variable). The subpopulations with pT3–4 disease and cT3 disease and with positive surgical margins showed a significant linear relationship between iXip and tumor volume.

Conclusion: We found elements supporting a possible correlation between iXip and aggressive PCa in terms of Gleason Score ≥ 7 and tumor volume $\geq 2.5 \text{ mm}^3$.

Keywords: iXip, biomarkers, PSA, PSA-IgM, prostate cancer

INTRODUCTION

Prostate cancer (PCa) is the most commonly diagnosed cancer in men in Italy with 36,074 cases in 2019 (18.5% of the total tumor diagnosis in Italy) (1); the most frequent in males aged in the range between 50–69 and over 70 years (1).

Almost all PCa-related deaths are due to the development of metastatic condition, which substantially remains an incurable disease (2–4). Accordingly, many efforts have been done to achieve an early diagnosis, mainly through the widespread diffusion of prostate specific antigen (PSA) and inherent diagnostic framework (5). However, the indiscriminate adoption of PSA-based strategies has also determined a substantial rate of diagnosis of clinically insignificant diseases and consequently exposed patients to overtreatment (6). Thus, various risk-stratification tools have been suggested in order to distinguish clinically relevant from insignificant PCa at diagnosis (7–10).

The iXip (Immune CompleX Predictive Index, Xeptagen, Venice, Italy) is a diagnostic integrated tool designed to improve the predictive performance of the PSA-IgM immune complexes only merging these data with serum PSA value, prostate volume, and patient's age (11, 12). The output generated by the algorithm is a numerical value ranging between 0% and 100% directly correlated with the risk of PCa at biopsy. A recent paper from our group (13) reviewed the actual clinical applications of this biomarker in biopsy naïve patients (11, 12, 14), in men with previous negative biopsy (15), and in patients with clinically significant cancers (12, 14) and during active surveillance (16). Based on the association of iXip with some biological features of PCa, we designed a prospective multicenter study (the Proxima trial) that aims to assess whether preoperative iXip can predict clinical relevance of PCa, as defined on pathological features available at radical prostatectomy specimen.

MATERIALS AND METHODS

Study Design and Participants

A prospective multicenter study was conducted from February 2018 to August 2019, the PROXIMA study (ClinicalTrials.gov identifier: NCT03413007). Three third-level urologic centers were involved after local ethical committee approval (protocol number 2969): the Spedali Civili Hospital of Brescia, the University Hospital of Parma, and the Papa Giovanni XXIII Hospital of Bergamo.

Between February 2018 and August 2019, all consecutive patients for radical prostatectomy to one of three urology departments located in North Italy (Brescia, Parma, and

Bergamo) were screened for possible involvement in the present study; each patient signed an informed consent. The inclusion criteria were as follows: patients with histologically proven prostate cancer, patients scheduled for radical prostatectomy, and able to provide consent and aged between 18 and 80 years. The exclusion criteria included the following: patients treated with neoadjuvant hormone therapy, salvage radical prostatectomy, with concomitant solid or hematological tumors, autoimmune disorders, immunosuppressive therapies, acute bacterial, or viral infections.

Variables

Baseline, clinical, and pathological outcomes, including Gleason Score, ISUP grading group, TNM stage, prostate volume, and tumor volume, were collected anonymously.

We considered the correlation between iXip and the following outcomes as primary endpoints:

- the pathological Gleason Score ≥ 7 or the pathological ISUP GG ≥ 3
- the pathological tumor volume $\geq 2.5 \text{ mm}^3$
- the pathological TNM pT stage ≥ 3 or pN > 0

As secondary endpoints, we evaluated the correlation between iXip and

- the clinically significant PCa (CS-PCa): tumor volume $\geq 2.5 \text{ mm}^3$ and pISUP ≥ 3 and pT ≥ 3
- the tumor residual (R1): PSA ≥ 0.1 or positive surgical margins

Data Sources/Measurements

The diagnosis of PCa was based on prostate biopsy done according to the protocol adopted by each institution, in general magnetic resonance imaging (MRI), while in a minority of cases the biopsy was done according to standard template (mapping). The indication to surgery was founded on international guidelines (17) and established by clinicians at each institution, generally after multidisciplinary discussion. Surgery was conducted through robotic, open, or laparoscopic approaches, according to the preference of the referring surgeon. Pathological examination was done at each institution by expert uro-pathologists, blinded of iXip evaluation.

Prostate volume was determined as a common procedure preceding the prostate biopsy by transrectal ultrasound using the ellipsoid formula (Volume = Length \times Height \times Width $\times \pi/6$) (18). The tumor volume was calculated by the expert local uro-pathologist as the percentage of prostatic involvement by cancer using specific software that evaluated the area covered by the entire tumor foci manually marked on every microsection (19). To calculate the volume in cubic centimeters (cm^3), the equation offered in the

study by Humphrey et al. (20) was applied: tumor volume (cm^3) = % of tumor volume \times number of sections \times 0.567 (conversion factor).

iXip was calculated for each patient using the online calculator <http://iXip.xeptagen.com> considering the initial PSA at biopsy, the PSA-IgM, the patient's age, and the prostate volume. After patient enrollment, during the routine blood collection, an aliquot of blood was reserved for the PSA-IgM assay, according to the procedures below described. Briefly, serum was obtained from the sample, frozen at -20°C in aliquots of 500 μl each, and sent for the centralized measurement to the Pharmacology Section of the University of Brescia. The expression level of PSA-IgM was measured in duplicate (100 μl /sample), using the XEPTAGEN Prostate-IC Kit (Code XG007, Xeptagen SpA, Venice, Italy), following the manufacturer's instructions. Absorbance was measured by an EnSight Multimode Plate Reader (PerkinElmer Italia, Milan, Italy) at 450 nm. The immunocomplex concentration of the patient samples was read directly on the x-axis by interpolating the absorbance on the standard curve. The PSA-IgM concentration was expressed in arbitrary units (AU/mL), within a linear range from 6.5 to 100 AU/mL. Sample size was calculated basing on data reported on iXip by Gallotta et al. (11) and considering appropriate to recruit at least 76 patients per center, for a total of at least 228 patients.

Quantitative Variables and Statistical Methods

Categorical variables were summarized as the absolute and relative frequencies, and numerical variables are shown as the median and IQR. For each binary outcome, using stepwise selection the significant predictors were first selected from the following set of covariates: age, Age Adjusted Charlson Comorbidity Score, PSA at diagnosis, prostate volume at the preoperative transrectal ultrasound, number of positive specimens at the prostate biopsy, average % specimen involvement, max % specimen involvement, digital rectal examination, clinical Gleason Score, clinical ISUP, clinical stage T, and clinical Stage N. The selected predictors were then used to estimate two multivariable logistic regression models for each outcome, the reference model with only the set of predictors and the model using the same set, with the addition of iXip. The prediction accuracy of the two models was assessed calculating the partial area under the receiver operating characteristic (ROC) curve, or pAUC (21). The pAUCs were compared using the bootstrap test implemented in the pROC package for R (22). Differences with p-values < 0.05 were considered statistically significant.

Statistical analyses were performed using Stata 16.1 (StataCorp. 2019, College Station, TX) and R 4.0.3 (R Core Team 2020, Vienna, Austria).

RESULTS

A total of 235 patients fulfilling inclusion criteria were enrolled. Population baseline and clinical and pathological features are

summarized in **Table 1**. The pathological Gleason Score (pGS) ≥ 7 was present in 179 (76%) patients; the pathological International Society of Urological Grading Group (pISUP GG) ≥ 3 was found in 82 (35%); and at pathological examination, the median tumor volume was 4.8 mm^3 (2.5–8.0) and 61 patients (26%) had a TV $< 2.5 \text{mm}^3$, 174 (74%) $> 2.5 \text{mm}^3$.

The pathological T2 (pT2) stage was found in 162 (69%) patients while the pT ≥ 3 in 73 (55%) patients; 14 patients (10%) had a lymph nodal invasion out of a total of 142 lymph node dissection performed. In clinically significant PCa (CS-PCa), a composite outcome including tumor volume $\geq 2.5 \text{mm}^3$ and pISUP GG ≥ 3 and pT ≥ 3 was present in 195 (83%) patients.

The tumor residual R1 intended PSA $\geq 0.1 \text{ng/ml}$ pT $>3a$ or pathological N+(pN+) or positive surgical margins (PSM) was found in 79 (34%) patients.

Relationship Between iXip and Gleason Score/ISUP Grading Group

We started considering the association between iXip (categorized into tertiles) and pGS. The number of patients with pGS ≥ 7 in the lowest tertile (iXip < 0.33) was 54/79 (68%), in the middle tertile (iXip between 0.33 and 0.45) was 67/78 (86%), and in the highest tertile was 58/78 (74%). Fisher's exact test showed a significant difference between the three groups, $p = 0.03$.

The rates of patients with pISUP GG ≥ 3 were 22/79 (28%), 35/78 (45%), and 25/78 (32%), respectively. No statistically significant differences were found between the three groups, $p = 0.07$.

Considering the middle tertile as the reference group, patients in the lowest tertile showed a significant lower risk of pGS ≥ 7 (OR = 0.4, 95% CI 0.2–0.8, $p = 0.01$) and of pISUP GG ≥ 3 (OR = 0.5, 95% CI 0.2–0.9, $p = 0.03$). No significant differences were found in the highest tertile in terms of pGS ≥ 7 (OR = 0.5, 95% CI 0.2–1.1, $p = 0.08$) and pISUP GG ≥ 3 (OR = 0.6, 95% CI 0.3–1.1, $p = 0.1$).

Investigating the ability of iXip to identify patients with pGS ≥ 7 , we found that the area under the ROC curve of the reference logistic model without iXip was 0.85; the addition of iXip to the model only slightly increased the AUC to 0.86 ($p = 0.2$). Comparing the partial AUCs of the two models in the interval with specificity between 0.6 and 1, we found a statistically significant difference (0.28 vs. 0.30; $p = 0.02$) (**Figure 1**).

Relationship Between iXip and Tumor Volume

Considering the tumor volume as a continuous variable, we found a significant linear relationship between iXip and the tumor volume in the subpopulation with pT3-4 disease, with a slope of 14.1 (95% CI 3.1–25.2, $p < 0.001$) and a correlation of 0.4 ($p < 0.001$), while for pT2 patients the slope was 0.6 (95% CI -3.3–4.6, $p = 0.8$) with a correlation of 0.03 ($p = 0.7$) (**Figure 2**). The slopes of pT2 and pT3-4 groups were significantly different ($p = 0.004$).

TABLE 1 | Baseline patients' characteristics: continuous variables were summarized as median and interquartile range, categorical variables as absolute and relative frequencies.

Variable	
Age (years)	67 (61–70)
Body mass index (kg/m²)	25.7 (23.7–27.7)
Charlson Comorbidity Score	
1	5 (2.1)
2	43 (18.3)
3	75 (31.9)
4	54 (23.0)
5	36 (15.3)
6	21 (8.9)
ASA score	
1	35 (14.9)
2	165 (70.2)
3	34 (14.5)
4	1 (0.4)
Digital rectal examination	
Positive	58 (24.7)
PSA at diagnosis ng/mL	7.05 (5.43–9.68)
Prostate volume ultrasound (mm³)	40.0 (30.0–57.5)
No. of positive biopsies	4.0 (2.0–6.0)
Average % specimen involvement	30.0 (15.0–48.4)
% max specimen involvement	50.0 (24.5–80.0)
Clinical Gleason Score	
6	83 (35.5)
7–8–9	151 (64.5)
Clinical ISUP	
1–2	170 (72.3)
3–4–5	65 (27.7)
PIRADS V2	
≤ 4	124 (68.9)
>4	56 (31.1)
PIRADS dimension (mm)	12.0 (8.0–15.3)
Clinical T stage	
cT1	89 (37.9)
cT2–cT3	146 (62.1)
Clinical N stage	
Pos	6 (3.0)
LAD	
Yes	142 (60.5)
Pathological T stage	
pT2	162 (68.9)
pT3–pT4	73 (31.1)
Pathological Gleason score	
6	56 (23.8)
7–9	179 (76.2)
Pathological ISUP	
1–2	153 (65.1)
3–5	82 (34.9)
Pathological N stage	
x	93 (39.5)
0	128 (54.5)
1	14 (6.0)
PSM	
Yes	59 (25.1)
Prostate volume (histological) (mm³)	40.0 (30.7–54.0)
Tumor volume (mm³)	4.8 (2.5–8.0)
≤2.5 mm ³	61 (26.0)
>2.5 mm ³	174 (74.0)
Lymph node yields	13.0 (9.0–19.0)
Positive lymph nodes	
Yes	14 (9.9)
PSA < 0.1 ng/ml at 40 p.o. day	

(Continued)

TABLE 1 | Continued

Variable	
Yes	213 (90.6)
Tumor residual	
Yes	156 (66.4)
PSA IgM (arbitrary units/mL)	185.95 (97.65–370.35)
iXip	0.40 (0.31–0.50)
<0.3	54 (23.0)
0.3–0.5	123 (52.3)
0.5–0.8	49 (20.9)
0.8–1	9 (3.8)

A significant association between iXip and tumor volume was also found in the clinical T3 (cT3) subpopulation (OR 16.4, 95% CI 3.2–30, p = 0.02).

The subpopulation with positive surgical margins showed a significant relationship between iXip and tumor volume with a slope of 13.2 (95% CI 3.2–23.2, p = 0.01) and a correlation of 0.4 (p = 0.004), while in the group with negative surgical margins the slope was 2.2 (95% CI 2.3–6.7, p = 0.3) with a correlation of 0.06 (p = 0.4) (Figure 3). The two slopes were significantly different (p = 0.03).

DISCUSSION

In this study, we noticed a relationship between iXip and the PCa biological aggressiveness; patients with iXip < 0.33 had a lower risk of pGS ≥ 7 and pISUP GG ≥ 3 considering, as reference tertile, iXip between 0.33 and 0.45.

The design of the current study goes above and beyond the previous research, investigating in depth the predictive ability of the PCa biological aggressiveness from a different perspective. iXip has been evaluated to account for comprehensive clinical data (including MRI) and bioptic features, referring to the description of PCa as a final pathology of radical prostatectomy.

Describing the proportion of patients with significant PCa in the different iXip tertiles, we observed a bell distribution for both the rates of pGS ≥ 7 and pISUPGG ≥ 3 which were higher in the middle tertile of iXip (0.33–0.45) than the lowest tertile (iXip < 0.33) and the highest tertile (iXip > 0.45). A possible explanation to this distribution could be found in how iXip was designed: the algorithm was developed in order to optimize the ROC curve at its ends rather than to obtain the best curve based on the highest value of AUC (11).

We acknowledge that the definition of tumor aggressiveness by pGS ≥ 7 is prone to criticism, but the ability of iXip resulted to be significant in terms of pAUC in the ROC curve only in the pGS > 7 cohort. These results supplement previous study results, based on prostate biopsy, which demonstrated the cutoff 0.3 for GS ≥ 7 disease (12, 14).

Tumor volume represents a key role by defining significant prostate cancer (19, 20), and EAU guidelines recommend to incorporate multivariable clinical risk-prediction tools into the decision-making process (17). The role of iXip as a predictive factor of tumor volume in cT3 tumors, confirmed after surgery in

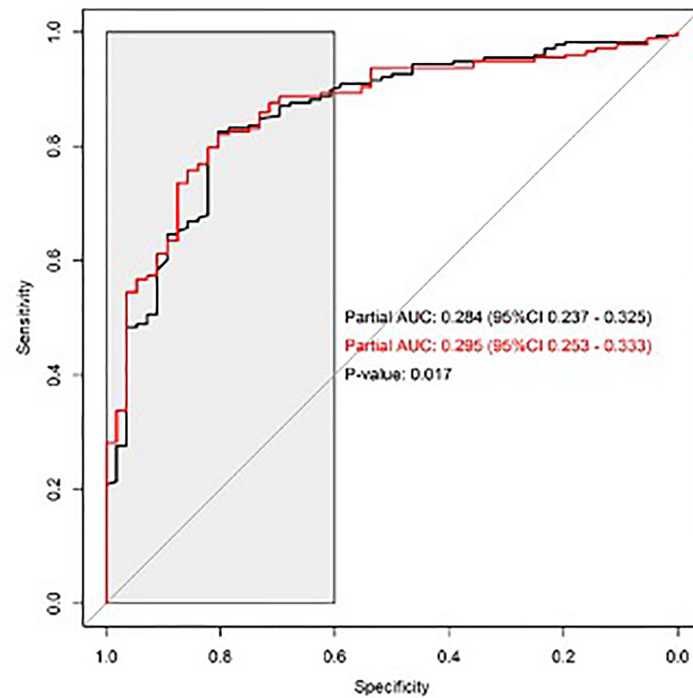


FIGURE 1 | Predictive ability of iXip for the identification of patients with pathological Gleason Score ≥ 7 ; ROC curves of the reference model without iXip (black line) and of the reference model with iXip (red line); the gray area indicates the region where the two partial AUCs were calculated and compared.

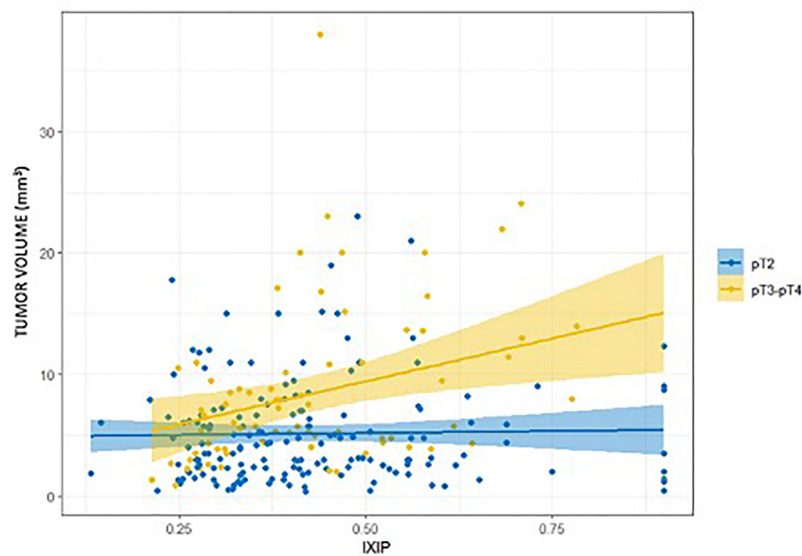


FIGURE 2 | Relationship between iXip and tumor volume in the pT2 and pT3–pT4 subgroups.

the pT3–4 and PSM subgroup, opens a possibility to integrate MRI variables in a new algorithm in order to better define the clinical tumor volume, further optimizing the diagnostic performance of iXip.

As a limitation of our analysis, this marker was used in a for-cause cohort of men already selected for radical prostatectomy and may perform differently in a screening setting where the prevalence of prostate cancer is lower.

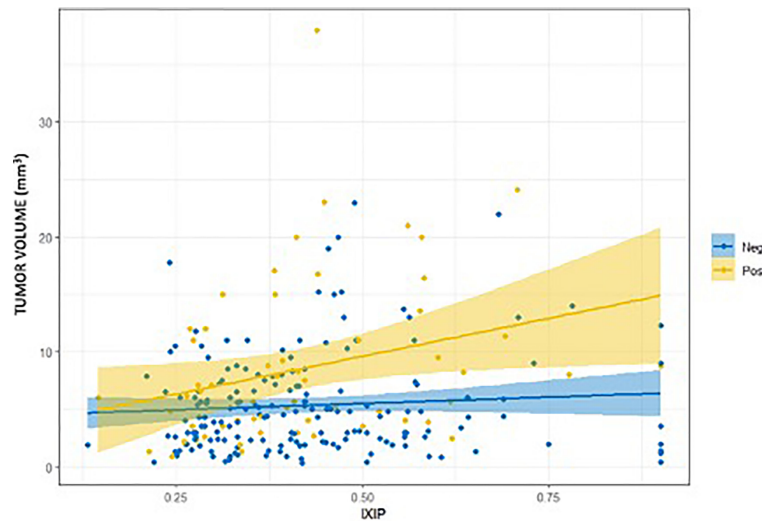


FIGURE 3 | Relationship between iXip and tumor volume splitting in the subgroups of patients with positive (Pos) and negative (Neg) surgical margins.

The strengths of our study include the multicenter, prospective study design in which all participants underwent radical prostatectomy for histological evaluation.

CONCLUSIONS

We provided evidence supporting a possible correlation between iXip and aggressive PCa at final pathology even in terms of Gleason Score ≥ 7 and ISUP ≥ 3 , according to the previous studies, and in addition, we demonstrated an interesting proportional relationship with tumor volume, in particular in the cT3, pT3–4, and PSM subgroups.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Brescia ethical committee, Brescia, Italy. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception: SFr, AAn, CS, SS, SFe, LdP. Interpretation or analysis of data: SFr, AAn, CS, SS, MS, SC, CL, AAb, EDM, CP, LA, SB, AIP, MR. Preparation of the manuscript: SFr, CL, AAn, MS. Revision for important intellectual content: AAn, MS, SS, AAb. Supervision: AAn, AnP, CS, SS, AG, SFe, LdP, MR. All authors contributed to the article and approved the submitted version.

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