UTILITY OF P63 IMMUNO-EXPRESSION IN DISTINGUISHING BENIGN MIMICKER LESIONS FROM ADENOCARCINOMA PROSTATE-AN OBSERVATIONAL ANALYTICAL STUDY

Rishabh Chaudhary, Pradeep Tandon, Pooja Jaiswal, Nausheen Sanaullah Khan

Department of Pathology

Integral Institute of Medical Sciences & Research, Integral University, Lucknow, U.P., India-226026.

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Address for correspondence

Dr. Pradeep Tandon
Department of Pathology
Integral Institute of Medical Sciences & Research, Lucknow, U.P., India-226026.
Email: tandonp72@yahoo.co.in
Contact no: +91-8840280751

ABSTRACT

In men, prostate cancer (PCA) is the second most common malignancy and is the sixth leading cause of death amongst them. Differentiation of benign mimicker lesions (BML) of the prostate from PCA can be challenging sometimes, especially when limited amount of tissue is available. Several immuno-histochemical (IHC) markers are recommended for distinguishing such lesions; which becomes expensive for the patient. The present study was planned to study the utility of P63 IHC marker for diagnosis of benign, BML and adenocarcinoma of the prostate (ACP). The present - observational and analytical study - was conducted on 50 biopsy specimens of the prostate in the Department of Pathology at IIMSR, Lucknow during the period January 2017 to September 2022. The lesions included were: 20 specimens of benign prostatic hyperplasia (BPH), 17 of BML, 3 of prostatic intraepithelial neoplasia (PIN) and 10 of PCA. In the BML group, 5 specimens of basal cell hyperplasia (BCH), 4 specimens of simple atrophy (SA), 3 specimens of post atrophic hyperplasia (PAH) and 5 specimens of adenosis were studied. In the present study, all specimens of BPH and BML are positive for P63 expression on IHC staining. In the PIN group, 2 out of 3 specimens were positive for P63. All 10 specimens of adenocarcinoma prostate were negative for P63 expression. We conclude that use of P63 IHC staining is adequate for distinguishing benign and BML of prostate from ACP in majority of the specimens.

KEYWORDS: P63, Benign mimicker lesions, Prostate carcinoma.

INTRODUCTION

Diseases of the prostate are common disorders among men. Statistics from across the world indicate that PCA is the second the most common malignancy and the sixth leading cause of death in males (1). In males, PCA constitutes 6.78% of all malignancies. In India, PCA is the third most common cancer in males (2). Diagnostic confirmation of PCA is heavily dependent on histological examination, and is based on architectural pattern of the lesion, presence of nuclear atypia and lack of basal cells. In some specimens, it is difficult to distinguish BML and premalignant lesions of the prostate from PCA - especially when limited amount of tissue is available for histopathological examination. Such a scenario can be seen with needle biopsies of the prostate in association with ambivalent serum marker studies. Some of the problem areas are: distinction of well differentiated ACP from BML like SA, atypical adenomatous hyperplasia (AAH), basal cell hyperplasia (BCH), atypical small acinar proliferation (ASAP) and high grade PIN (3). Diagnosis of ACP can be difficult when only a small focus of malignancy is present in the biopsy. IHC has been employed to distinguish BML and premalignant lesions of prostate from ACP. Absence of basal cell markers along with presence of prostate cancer associated markers - like AMACR - is commonly employed in making the distinction. However, positive AMACR staining is not seen in all specimens of ACP. Conversely, negative AMACR staining does not rule out malignancy. AMACR staining may be absent in 5% to 25% patients of ACP (4). It can be negative in 30% of patients with atrophic carcinoma, in 32% to 38% patients of foamy gland carcinoma, and in 23% to 30% patients of pseudo-hyperplastic variant of ACP (5). Moreover, positive AMACR staining can be seen in some specimens of involvement of the prostate gland by secondary tumors like urothelial carcinoma and colonic adenocarcinoma (6). Positive AMACR expression can also be seen in some specimens with high grade PIN (7) and AAH (8). Fatima et al found AMACR positivity in two out of 44

specimens of BPH (9). Similar results are reported by Jiang et al (10). Evans et al found that BML like AAH, SA, PAH and BCH may show positive AMACR staining in some instances (11). In view of the above limitations of AMACR staining, present study was planned to study the role of P63 immunostaining in distinguishing non-neoplastic, BML and malignant lesions of the prostate.

MATERIAL AND METHODS

The present study was conducted in the Department of Pathology at Integral Institute of Medical Sciences and Research (IIMS&R), Lucknow, India. The study protocol was approved by the Institutional Research Committee and Institutional Ethical Committee (IEC/IIMS&R/2021/35). 50 prostatic tissue biopsy specimens received for histopathological examination, during the period January 2017 to September 2022, are included in the study. Prostatic biopsy specimens of ACP from patients on adjuvant chemo/radiotherapy were excluded from the study.

After collection in 10% neutral buffered formalin, the biopsy specimens were processed as per the standard protocol. Histological slides were stained by Hematoxylin and Eosin stain. All biopsies showing BPH, BML or ACP were subjected to immunohistochemical demonstration of P63 antigen by PAP technique. Rabbit monoclonal antibody (RAB) against P63 was the primary antibody used (clone DBR16.1, Diagnostic Biosystems) in the PAP technique. Heat retrieval of antigen was done on Thermo scientific PT module using Tris buffer (pH=9) for 20 minutes at 95 C°. A benign breast lesion was used as positive control. Slides were evaluated for P63 staining. Positive stain was visualized as brown nuclear staining. After counting P63 positive cells in at least 3 different areas of the biopsy, the mean percentage of cells showing positive staining were estimated. A 3-point scoring scale was used for representing P63 positivity. Less than 5% staining was scored as 0, between 5 to 25% as 1+, between 25 to 75% as 2+ and over 75% as 3+. Scoring of staining intensity cells was also done on a 3-point scale as follows: 1+ for weak staining, 2+ for moderate staining and 3+ for strong staining. Total staining score was obtained by multiplying mean percentage of cells with positive staining by staining intensity score.

RESULTS

Out of 50 specimens included in our study, there were 20 specimens of BPH, 17 specimens of BML, 3 specimens of PIN and 10 specimens of invasive ACP. Amongst BML, 5 specimens of BCH, 4 of SA, 3 of PAH and 5 specimens of adenosis were seen.

The age-wise distribution of different lesions seen in our study is shown in table 1.

Age in years	BPH	Mimicker lesions	PIN	PCA
40 - 50	0	01	0	01
51 - 60	05	07	02	03
61-70	08	06	01	03
71-80	06	03	0	03
81-90	01	0	0	0
Total	20	17	03	10

Table 1: The Age-wise Distribution of Different Lesions in our Study

Majority of patients with BPH are seen in the age-range of 61- 80 years (70 % of all specimens). BML were seen predominantly in the age-range of 51-70 years (65 % of all specimens). ACP was most commonly present in patients between the age-range of 51-70 years (65% of all specimens).

Gleason score of invasive ACP included in our study is shown in table 2. As seen from the table, majority of ACP in our study were high grade carcinomas.

Gleason Score	PCA (n)	Percentage (%)
7	2	20%
8	3	30%
9	5	50%
10	0	0%
Total	10	100%

Table 2: Gleason Score of PCA in our Study

P63 expression in different lesions of the prostate is shown in table 3. All biopsies showing BPH and BML were positive for P63. In patients with PIN, P63 positivity was seen in 2 out of 3 specimens. All biopsies of ACP were negative for P63.

P63 expression	BPH (n)	Mimicker lesions (n)	PIN (n)	PCA (n)
Negative	0	0	1	10
Positive	20	17	2	0
Fisher' exact test value is 0.0001. The result is significant at $p < 0.05$				

Table 3: P63 Expression in Different Lesions of Prostate

DISCUSSION

Cancer of the Prostate gland accounts for 9.7% of all malignancies in men. It is the sixth leading cause of death due to cancer amongst them. PCA is the third most common cancer in men in India, constituting 6.78% of all malignancies. In majority of the patients, a histopathological examination of the prostatic biopsy is done for confirmation of the clinical diagnosis. However, in some instances, differentiation of BML of the prostate from malignancy is difficult on routine histopathological examination. Important BML that can be misdiagnosed are: SA, adenosis (also called atypical adenomatous hyperplasia or AAH), BCH and atypical small acinar proliferation (ASAP) (3). In a recent study, it was seen that amongst the above-mentioned lesions, atrophy and partial atrophy are commonly misdiagnosed as prostatic malignancy (12). Nephrogenic adenoma, mesonephric hyperplasia and seminal vesicular tissue can also be confused with ACP. This dilemma is especially relevant when limited amount of tissue is available for histopathology - as in core needle biopsies. To overcome this problem, immunohistochemistryis being employed with increasing frequency. As per recommendation of International Society of Urological Pathology (ISUP), IHC is not needed in obvious examples of ACP or benign disorders. However, in suspicious specimens - to conserve tissue and the suspicious tissue focus - they recommend a combination of IHC staining with basal cell and prostate cancer cell specific markers, employing a cocktail of three different antibodies with different chromogens. However, in view of existing facilities in our country, where triple cocktail marker is expensive and not easily available, judicious use of a single IHC marker may be a preferable option. Some of the markers commonly used for delineating basal cell are P63 and high molecular weight cytokeratin (HMWCK). According to Signoretti et al, P63 expression is seen in all prostatic biopsies having presence of basal cells (13). Singh V et al found P63 to be a useful marker having sensitivity and specificity of 90% and 100% respectively (14).

In our series, P63 expression is seen in all specimens of BPH. This concurs with results seen in some other studies (Table 4).

In our study, as shown in Table 3, all patients with BML of the prostate were P63 positive. P63 positivity of 25-75% cells were seen in all specimens of BCH (5/5 biopsies) and SA (4/4 biopsies. In patients with PAH and atypical adenosis, positive staining was seen in all specimens, but was focal in nature (in < 25% cells).

In the study by Kalantari et al, out of 12 biopsies with adenosis, 8 showed P63 positivity in 5-75% of the cells. In remaining 4 specimens, P63 positivity was seen in less than 5% of the cells. In the same study, out of 16 biopsies with partial atrophy, 6 biopsies showed P63 positivity in 5-75% of cells. In the remaining 10 specimens, positivity was seen in less than 5% of the cells. Parson et al also found focal positivity in prostatic atrophy (21). In the study by Wang et al, 30% biopsies with partial atrophy were negative for P63 (22).

In our series, in biopsies showing HGPIN, P63 positivity was seen in 2 out of 3 specimens. In a study by Kalantari et al, P63 positivity was seen in all specimens of HGPIN (17). Parson et al found focal expression of P63 in HGPIN. However, Lu et al. found P63 positivity in 86.67% of specimens with HGPIN (23).

In the present study, none of the biopsies of ACP showed P63 expression. Our findings are in conformity with some other studies as shown in table 5.

Sr. no.	Study	BPH (n)	Positive P63 expression (n)	%age with positive staining
1.	Present study	20	20	100%
2.	Ibrahim et al (15)	17	17	100%
3.	Premalatha et al (16)	15	14	94%
4.	Kalantari et al (17)	20	20	100%
5.	Koshy et al (18)	102	102	100%
6.	Ng VW et al (19)	138	128	95.3%
7.	Quatani FA et al (20)	50	49	98 %

Table 4: P63 Expression in BPH in Different Studies

Sr. no.	Author	Total no of specimens	Positive P63 expression	Specimens with positive staining
1.	Present study	10	0	0%
2.	Kalantari (17)	38	0	0%
3.	Ibrahim (15)	30	0	0%

Table 5: P63 Expression in PCA

4.	Grisanzio (24)	130	04	3%
5.	Koshy (18)	28	0	0%
6.	Quatani FA (20)	50	02	04%
7.	Ng VW (19)	113	09	6.2%

Table 5: P63 Expression in PCA

Thus, our study and similar studies by other workers show that use of a single marker - P63 - can resolve majority of diagnostic dilemmas concerning BML in prostatic biopsies. In occasional specimens, if doubt persists regarding the exact diagnosis, triple marker study can be initiated. This approach is economically more viable in resource poor settings.

Limitations of the study

Limited number of specimens and lack of repeat biopsy and follow up data of the patients with mimicker lesions.

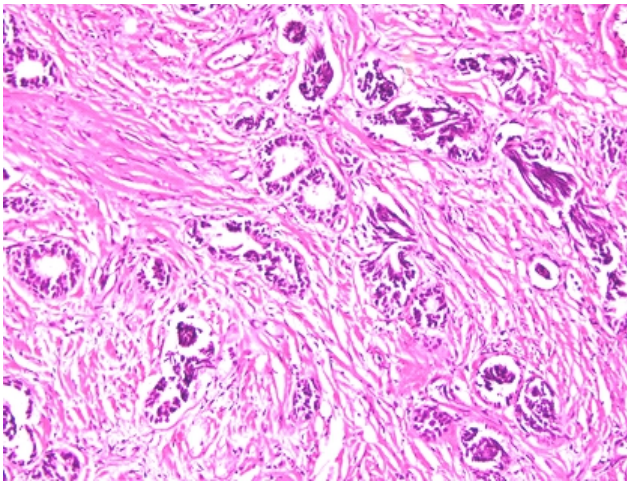

Fig. 1: H&E Stained section showing Atrophic Glands with Infiltrative Features 200x

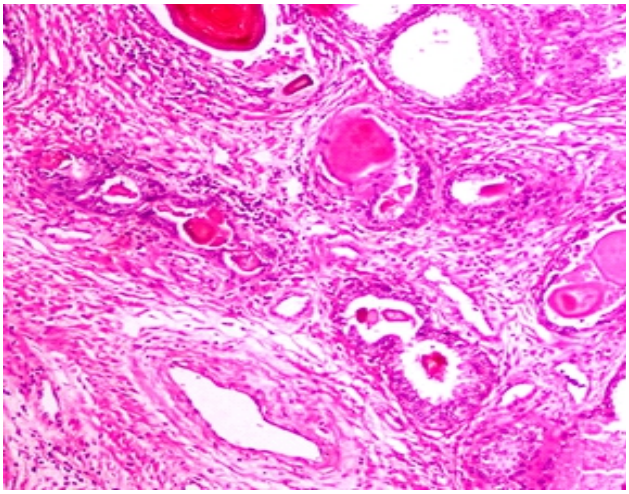

Fig. 3: H&E stained section showing presence of infiltrating atypical glands

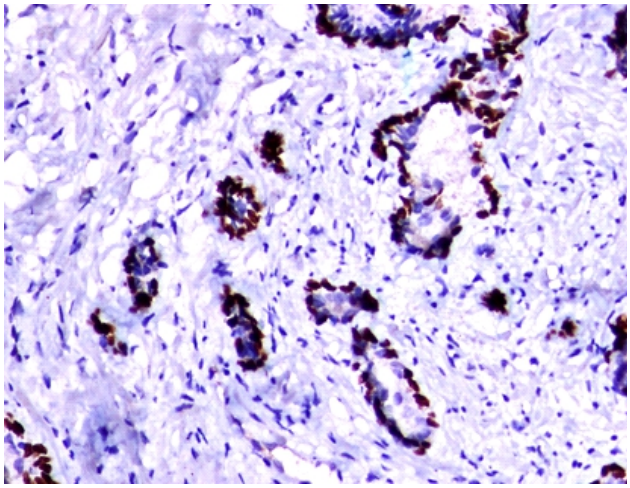

Fig. 2: Same Case as in fig 1 showing Positive Staining for P63

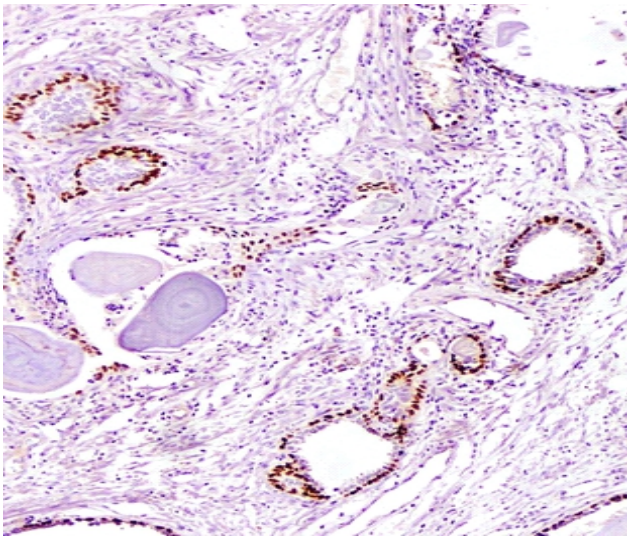

Fig. 4: Same case as in fig 3 showing P63 positivity

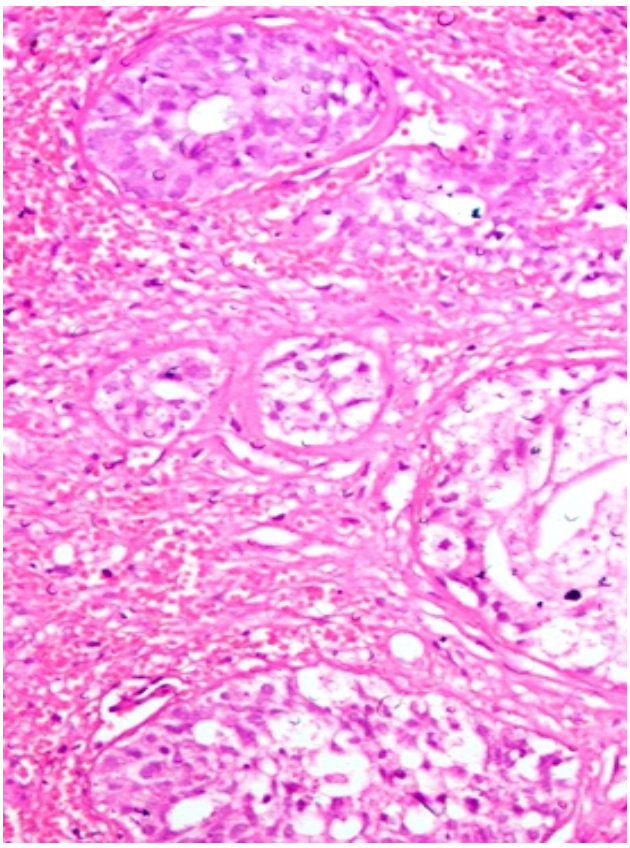

Fig. 5: Showing basal cell hyperplasia and a gland with cribriform hyperplasia

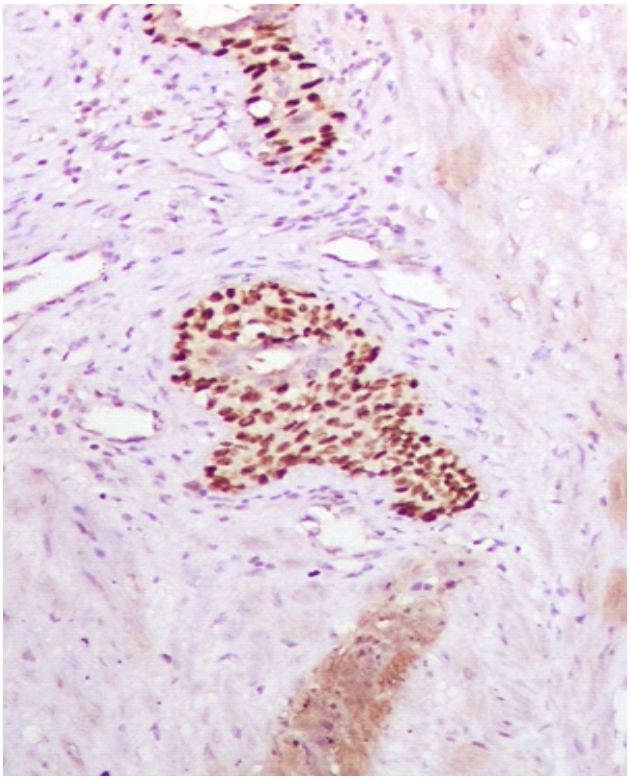

Fig. 6: Same case as in fig 5 showing P63 positivity

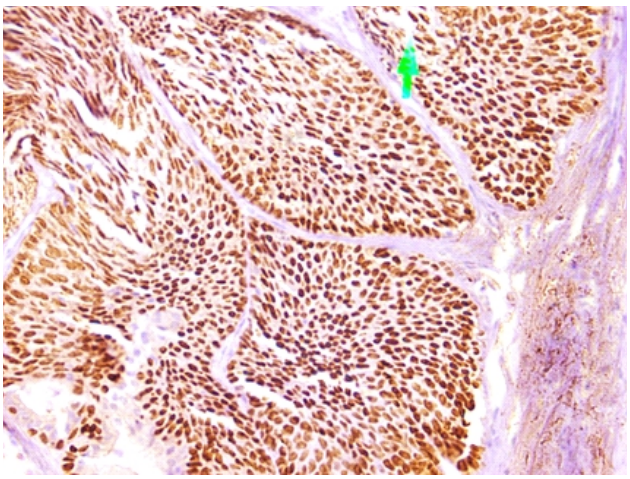

Fig. 7: A case of BPH with Focal Areas of Marked Basal Cell Hyperplasia Showing P63 Positivity

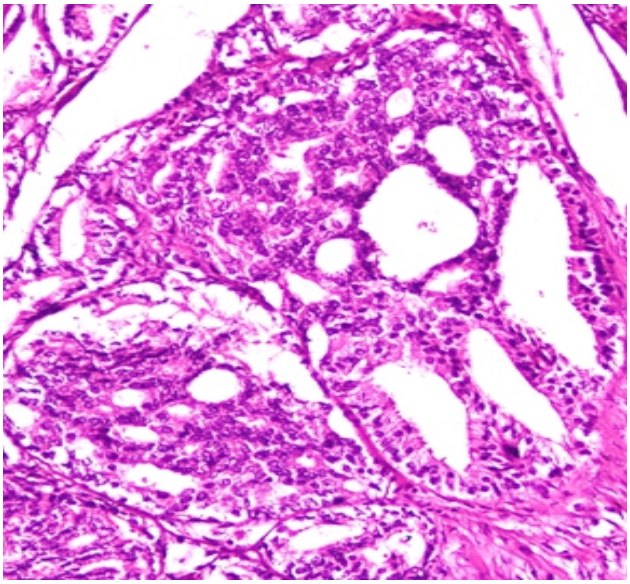

Fig. 8: A case of Adenocarcinoma of Prostate

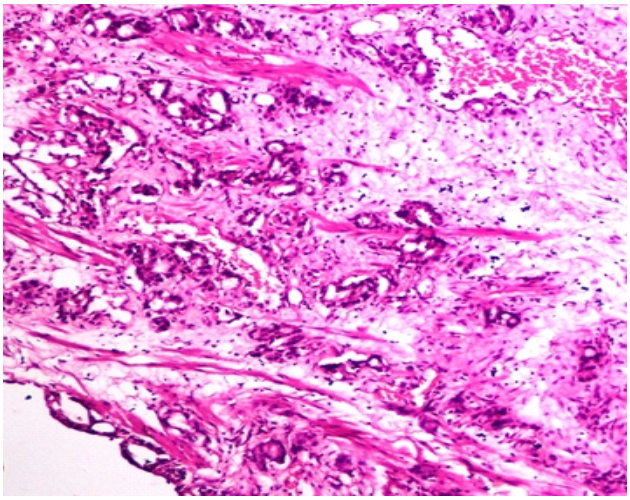

Fig. 9: Same Case as Figure 8 showing Features Resembling Atrophy as seen in fig 1

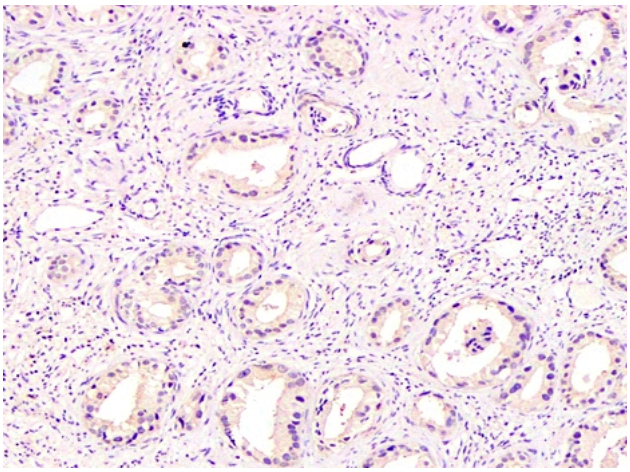

Fig. 10: Negative P63 staining of the case shown in fig 8

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Orcid ID:

Rishabh Chaudhary - https://orcid.org/0009-0009-2784-5154

Pradeep Tandon - https://orcid.org/0000-0002-4976-6707

Pooja Jaiswal - https://orcid.org/0000-0002-9441-3492

Nausheen Sanaullah Khan - https://orcid.org/0000-0001-5693-0061

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