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Original Article

Assessment of Correlation Between Histopathological Grading and Immunohistochemical Markers in Prostate Adenocarcinoma

Sharvan Kumar Bhargawa[1], Sweta Kanaujia[2], Vaishali Singh[3], Awadhesh Kumar[4]

[1]Associate Professor, Department of Pathology, Government Medical College, Budaun.
[2]Assistant Professor, Department of Pathology, Government Medical College, Budaun.
[3]Assistant Professor, Department of Immunohematology and blood transfusion, Government Medical College Budaun.
[4]Associate Professor, Department of Community Medicine, Rajarshi Dashrath Autonomous State, Medical College, Ayodhya, U.P.

Corresponding Author:

Awadhesh Kumar
Associate Professor, Department of Community Medicine, Rajarshi Dashrath Autonomous State, Medical College, Ayodhya, U.P.

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ABSTRACT

Background: Prostate adenocarcinoma is one of the most prevalent malignancies among men, with prognosis closely linked to histological grade and molecular alterations. This study aimed to evaluate the correlation between histopathological grading and immunohistochemical (IHC) expression of prostate-specific antigen (PSA), α-methylacyl-CoA racemase (AMACR), Ki-67, and p53 in prostate adenocarcinoma. **Material & Methods**: Formalin-fixed, paraffin-embedded tissue samples from diagnosed prostate adenocarcinoma cases were analyzed. Histological grading was performed using the Gleason/ISUP system. IHC staining for PSA, AMACR, Ki-67, and p53 was conducted, and their expression patterns were correlated with histopathological grades. **Result**s: PSA expression showed strong cytoplasmic positivity in low-grade tumors, decreasing significantly with higher Gleason grades ($p<0.05$). AMACR demonstrated increasing intensity and distribution with tumor grade, showing diffuse cytoplasmic staining in high-grade carcinomas. The Ki-67 proliferation index exhibited a positive correlation with histological grade, reflecting enhanced proliferative activity in poorly differentiated tumors. p53 positivity was observed in 40% of cases, predominantly in higher-grade lesions, indicating tumor aggressiveness. **Conclusion**: A significant correlation exists between histopathological grade and IHC markers in prostate adenocarcinoma. PSA and AMACR are valuable diagnostic markers, while Ki-67 and p53 provide crucial prognostic insights.

Keywords*: Prostate adenocarcinoma, Gleason grading, PSA, AMACR, Ki-67, p53, Immunohistochemistry, prognostic markers.*

INTRODUCTION

Prostate adenocarcinoma is one of the most common malignancies affecting men worldwide and remains a significant cause of cancer-related morbidity and mortality. It accounts for approximately 15% of all cancers diagnosed in men and represents the second leading cause of cancer death after lung cancer globally (1). The incidence of prostate carcinoma varies geographically, being highest in Western countries and lowest in Asia, largely due to differences in genetic predisposition, dietary habits, and screening practices (2). However, in recent years, there has been an increasing trend in the diagnosis of prostate cancer in developing countries, including India, owing to greater awareness, improved diagnostic modalities, and aging populations.

Histopathological examination of prostate tissue obtained by biopsy remains the gold standard for diagnosing prostate adenocarcinoma (3). The histological grading system most widely used for evaluating the aggressiveness of prostate cancer is the Gleason grading system, developed by Donald Gleason in the 1960s and later modified by the International Society of Urological Pathology (ISUP) (4). This system is based on the architectural patterns of tumor glands and categorizes the tumor into grades ranging from 1 to 5, where higher grades indicate poor differentiation and more aggressive biological behavior (5). The sum of the primary and secondary patterns gives the Gleason score, which serves as a powerful prognostic indicator for disease progression, metastasis, and patient survival.

Despite its reliability and widespread use, histopathological grading alone may not fully predict tumor behavior, especially in borderline or morphologically ambiguous cases. The inter-observer variability among pathologists, sampling errors, and tumor heterogeneity further complicate accurate prognostication (6). Therefore, there is an increasing interest in the use of immunohistochemical (IHC) markers as adjuncts to conventional histopathology for better characterization and prognostication of prostate adenocarcinoma.

Immunohistochemistry allows the visualization of specific proteins within tissue sections, aiding in identifying tumor origin, biological activity, and potential therapeutic targets. In prostate cancer, several markers have been extensively studied for diagnostic and prognostic purposes. Among these, Prostate-Specific Antigen (PSA), Prostatic Acid Phosphatase (PAP), Alpha-Methylacyl-CoA Racemase (AMACR), p63, and High Molecular Weight Cytokeratin (HMWCK) play critical roles in tumor identification (7). PSA and PAP are traditional markers indicating prostatic origin, whereas AMACR is a marker of malignancy, being upregulated in prostate adenocarcinoma cells (8). Conversely, basal cell markers like p63 and HMWCK are typically absent in malignant glands but present in benign prostatic tissue, thereby helping in the differential diagnosis of atypical glands (9).

In addition to diagnostic markers, several proliferation and tumor suppressor markers, such as Ki-67, p53, and Bcl-2, have been explored to correlate with tumor grade, aggressiveness, and prognosis (10). Ki-67, a nuclear protein expressed during active phases of the cell cycle, reflects the proliferative index of the tumor. Its expression has been shown to increase with higher Gleason scores and more advanced stages, indicating a potential role as a prognostic biomarker (11). Similarly, p53 mutation and Bcl-2 overexpression are associated with apoptosis resistance and poor prognosis in prostate cancer (12,13). The integration of these molecular markers with histological grading could thus refine prognostic stratification and guide therapeutic decisions more effectively.

Correlating histopathological grading with immunohistochemical markers can offer valuable insights into tumor differentiation, biological aggressiveness, and potential therapeutic targets. It can also aid in identifying early lesions that may progress to clinically significant cancers, improving patient management through individualized therapeutic approaches.

MATERIALS AND METHODS

Study Design and Setting

A retrospective observational study was conducted at a tertiary care teaching hospital in North India. The study included all histopathologically confirmed cases of prostate adenocarcinoma received during the study period of 1.5 years from January 2023 to August 2025. All prostate specimens diagnosed as adenocarcinoma on routine histopathological examination were included. These specimens comprised needle biopsies, transurethral resection of prostate (TURP) chips, and radical prostatectomy specimens. The relevant clinical and demographic details such as age, presenting symptoms, serum prostate-specific antigen (PSA) levels, and radiological findings were retrieved from medical records and requisition forms. A total of 50 histopathologically confirmed cases of prostate adenocarcinoma meeting the inclusion criteria were included in the final analysis. The sample size was determined based on the number of available cases during the study period according to convenient sampling technique and feasibility of performing immune-histochemistry.

Inclusion & Exclusion Criteria

Cases histologically diagnosed as prostate adenocarcinoma with available representative paraffin blocks with adequate tissue for performing immune-histochemical staining with complete clinical and histopathological data available were included in the study. However cases with inadequate or autolyzed tissue unsuitable for IHC evaluation or cases of Benign prostatic hyperplasia (BPH) or prostatic intraepithelial neoplasia (PIN) without invasive carcinoma and those with incomplete data or unavailable paraffin blocks were excluded from the study.

Histopathological Evaluation

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 4-µm thickness and stained with Hematoxylin and Eosin (H&E) using standard protocol. Each case was evaluated microscopically for:

- Architectural pattern and glandular differentiation
- Nuclear atypia, mitosis, and nucleoli prominence
- Presence of perineural invasion and necrosis

The grading was assigned according to the Modified Gleason Grading System (2014 ISUP) (14). For each case, primary and secondary Gleason patterns were recorded, and the Gleason score and Grade Group (1–5) were assigned accordingly.

Immunohistochemical Analysis

IHC was performed on representative sections using the polymer-based HRP detection system. The following primary antibodies were used:

Marker	Clone	Cellular Localization	Diagnostic/Prognostic Role
PSA (Prostate-Specific Antigen)	Polyclonal	Cytoplasmic	Confirms prostatic origin
p63	4A4	Nuclear	Basal cell marker (absent in carcinoma)
HMWCK (34βE12)	34βE12	Cytoplasmic	Basal cell marker for benign glands
AMACR (α-Methylacyl-CoA Racemase)	13H4	Cytoplasmic	Positive in carcinoma cells
Ki-67	MIB-1	Nuclear	Proliferation index
p53	DO-7	Nuclear	Tumor suppressor, associated with aggressiveness

Immunohistochemical Procedure

Sections (3–4 µm) were mounted on poly-L-lysine-coated slides, de-paraffinized in xylene, and rehydrated through graded alcohols. Antigen retrieval was done in citrate buffer (pH 6.0) using a pressure cooker for 15 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes. The slides were then incubated with the respective primary antibodies for 1 hour at room temperature, followed by secondary antibody and diaminobenzidine (DAB) chromogen visualization. Counterstaining was performed with hematoxylin, and slides were mounted with DPX. Known positive control slides were included with each batch. Negative controls were processed similarly but without the primary antibody.

Evaluation of Immunohistochemical Staining

Each IHC-stained slide was examined independently by two pathologists.

- **PSA, AMACR, and p53** were evaluated for intensity (weak, moderate, strong) and percentage of positive tumor cells.
- **Ki-67** labeling index was calculated as the percentage of positively stained nuclei among 1000 tumor cells in high-power fields.
- **p63** and **HMWCK** were recorded as either present or absent in basal cells.

The IHC expression scores were correlated with the Gleason score and Grade Group.

Statistical Analysis

All data was entered into Microsoft Excel and analyzed using SPSS software version 22.0.

Descriptive statistics were used for demographic data. The correlation between histopathological grading and immunohistochemical marker expression was evaluated using:

- Chi-square test or Fisher's exact test for categorical variables.
- Spearman's correlation coefficient (r) to assess the strength of association.
  A p-value < 0.05 was considered statistically significant.

RESULTS

1. Demographic and Clinical Profile

A total of **50 histopathologically confirmed cases** of prostate adenocarcinoma were analyzed. The **age of patients** ranged from **52 to 85 years**, with a **mean age of 68.4 ± 7.8 years**. The majority of patients (60%) were in the **61–70 years** age group, followed by 24% in the **71–80 years** range (Table 1). The most common presenting complaints were **lower urinary tract symptoms (72%)**, followed by **hematuria (18%)** and **bone pain (10%).** Serum **PSA levels** ranged from **6.2 to 150 ng/mL** with a **mean of 48.6 ng/ml.** A significant proportion of high-grade tumors (Gleason score ≥ 8) showed markedly elevated PSA (>50 ng/mL).

Table 1: Age Distribution of Study Subjects (n=50)

Age Group (years)	Number of Cases	Percentage (%)
51–60 yrs	6	12
61–70 yrs	30	60
71–80 yrs	12	24
>80 yrs	2	4
Total	**50**	**100**

2. Histopathological Grading (Gleason/ISUP System)

Based on the **2014 ISUP modified Gleason grading system**, the distribution of cases is shown in Table 2. The most common grade group observed was **Grade Group 2 (Gleason score 3+4=7),** comprising **34%** of cases, followed by **Grade Group 3 (Gleason score 4+3=7)** in **24%.** High-grade tumors (Grade Groups 4 and 5) constituted **28%** of cases, whereas well-differentiated tumors (Grade Group 1) were rare (14%).

Table 2: Distribution of Cases According to Gleason/ISUP Grade Groups

Grade Group	Gleason Score	Differentiation	Number of Cases	Percentage (%)
1	3 + 3 = 6	Well differentiated	7	14
2	3 + 4 = 7	Moderately differentiated	17	34
3	4 + 3 = 7	Moderately differentiated	12	24
4	4 + 4 = 8	Poorly differentiated	8	16
5	4 + 5 / 5 + 4 / 5 + 5 = 9–10	Poorly differentiated	6	12
Total	—	—	**50**	**100**

3. Immunohistochemical (IHC) Marker Expression

All cases were subjected to IHC analysis using the following panel: **PSA, AMACR, p63, HMWCK, Ki-67, and p53.**

3.1 PSA Expression

PSA positivity was observed in **46 (92%)** of cases. The staining was cytoplasmic, diffuse, and strong in most low- and intermediate-grade tumors. A decline in PSA expression intensity was noted with increasing Gleason grade (Figure 1).

3.2 AMACR Expression

AMACR showed **positive cytoplasmic staining** in **44 (88%)** cases. Its expression was more intense in higher-grade tumors, showing a positive correlation with Gleason score ($p = 0.021$).

3.3 Basal Cell Markers (p63 and HMWCK)

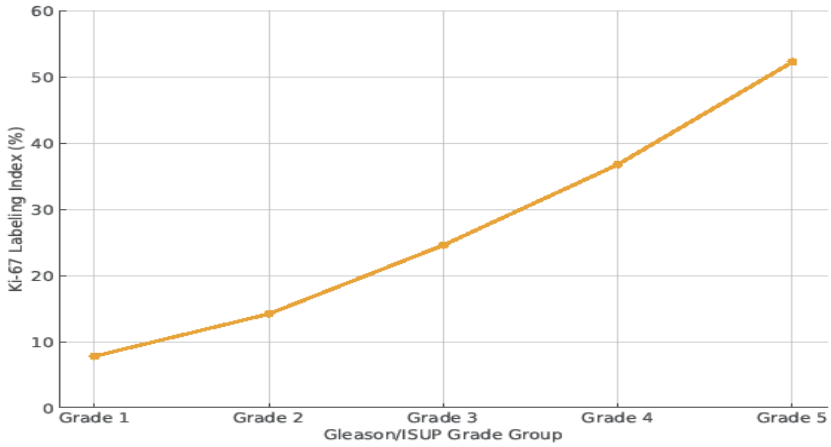
Basal cell markers **p63** and **HMWCK (34βE12)** were **negative in all adenocarcinoma cases,** confirming malignant transformation. However, internal controls (benign glands) showed strong nuclear (p63) and cytoplasmic (HMWCK) positivity, ensuring test validity.

3.4 Ki-67 Proliferation Index

The **Ki-67 labeling index (LI)** ranged from **5% to 60%,** with a **mean of 26.4%.** The proliferative index increased with higher Gleason grade, demonstrating a statistically significant positive correlation ($p < 0.001$). (Figure 2)

- **Grade Group 1:** Mean Ki-67 = 7.8%
- **Grade Group 2:** Mean Ki-67 = 14.2%
- **Grade Group 3:** Mean Ki-67 = 24.6%
- **Grade Group 4:** Mean Ki-67 = 36.8%
- **Grade Group 5:** Mean Ki-67 = 52.3%

Figure 2: Ki-67 Proliferation Index Across Gleason Grade Groups



3.5 p53 Expression

Nuclear p53 positivity was detected in **20 (40%)** of cases, predominantly in high-grade tumors (Grade Groups 4 and 5). The correlation between p53 positivity and Gleason grade was statistically significant ($p = 0.008$).

Table 3: Correlation of Immunohistochemical Markers with Gleason Grade Groups

Marker	Grade Group 1 (n=7)	Grade Group 2 (n=17)	Grade Group 3 (n=12)	Grade Group 4 (n=8)	Grade Group 5 (n=6)	p-value
PSA positive (%)	7 (100%)	17 (100%)	11 (92%)	7 (87.5%)	4 (66.7%)	0.041*

AMACR positive (%)	5 (71.4%)	15 (88.2%)	11 (91.6%)	8 (100%)	5 (83.3%)	0.021*
p63 positive (%)	0	0	0	0	0	—
HMWCK positive (%)	0	0	0	0	0	—
Ki-67 (Mean %)	7.8	14.2	24.6	36.8	52.3	<0.001**
p53 positive (%)	1 (14.3%)	3 (17.6%)	5 (41.6%)	6 (75%)	5 (83.3%)	0.008**

*p < 0.05 statistically significant; **p < 0.01 highly significant

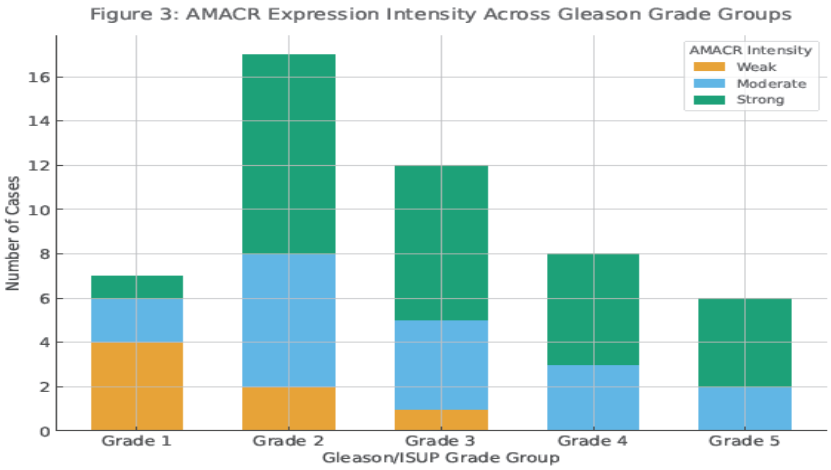


Figure 3: Stacked bar chart representing AMACR expression intensity (weak/moderate/strong) in different grade groups, highlighting stronger staining in higher grades.

4. Correlation Analysis

Using **Spearman's correlation coefficient**, significant positive correlations were found between **Gleason grade** and:

- **Ki-67 labeling index** (r = 0.72, p < 0.001)
- **p53 expression** (r = 0.61, p = 0.008)
- **AMACR intensity** (r = 0.46, p = 0.021)

A weak **negative correlation** was observed between Gleason grade and **PSA intensity** (r = –0.39, p = 0.041), indicating decreased PSA expression with dedifferentiation.

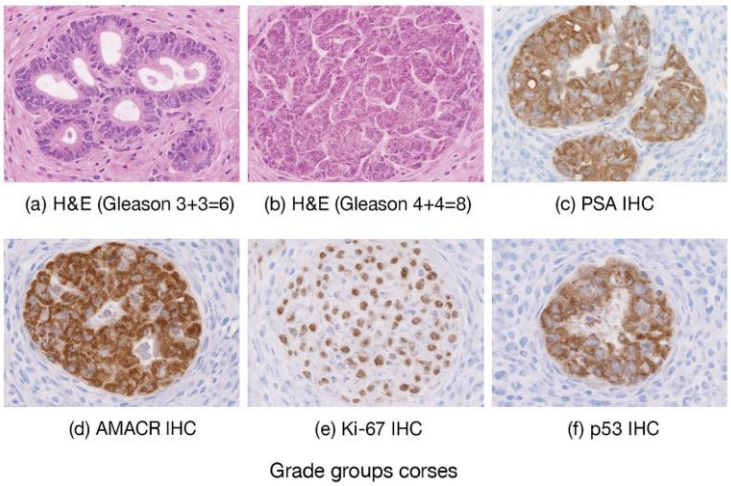


Figure 4:

Photomicrographs:

- (a) H&E section showing well-formed glands (Gleason 3+3=6) (×200).
- (b) Poorly formed cribriform glands (Gleason 4+4=8) (×200).
- (c) PSA IHC showing strong cytoplasmic positivity in low-grade tumor (×400).
- (d) AMACR IHC showing diffuse cytoplasmic staining in high-grade tumor (×400).

- (e) Ki-67 nuclear positivity in poorly differentiated carcinoma (×400).
- (f) p53 nuclear accumulation in Grade Group 5 tumor (×400).

These findings confirm that immunohistochemical markers—particularly **Ki-67 and p53**—can serve as reliable adjuncts to histopathological grading for assessing tumor aggressiveness and potential prognosis in prostate adenocarcinoma.

DISCUSSION

Prostate adenocarcinoma represents the most common malignancy of the male genitourinary tract, with prognosis and therapeutic decisions largely influenced by histopathological grade and molecular biomarker expression. The present study aimed to correlate histopathological grading, based on the Gleason/ISUP system, with immunohistochemical (IHC) markers including prostate-specific antigen (PSA), α-methylacyl-CoA racemase (AMACR), Ki-67, and p53. The findings reveal a consistent relationship between increasing histological grade and altered IHC profiles, suggesting that a combined morphologic and molecular approach can enhance diagnostic precision and prognostication.

In the current study, PSA expression showed strong cytoplasmic positivity in well-differentiated (low-grade) carcinomas, with a gradual decline in intensity and extent in higher-grade tumors. This inverse relationship aligns with reports by Shah et al. (2019), who demonstrated reduced PSA reactivity in poorly differentiated tumors, indicating loss of glandular differentiation with increasing tumor grade (15). Similar findings were reported by Varma et al. (2018) in an Indian cohort, where PSA negativity was observed in approximately 20% of high-grade carcinomas, complicating diagnosis in metastatic settings (16). The reduced PSA expression may reflect dedifferentiation of malignant cells and decreased secretory function, emphasizing the need for adjunctive markers in such cases.

AMACR, a mitochondrial and peroxisomal enzyme involved in β-oxidation of branched-chain fatty acids, has been validated as a sensitive diagnostic marker for prostatic carcinoma. In this study, AMACR expression increased with histological grade, showing diffuse and strong cytoplasmic staining in higher-grade tumors. This observation corroborates findings by Rubin et al. (2002), who first described AMACR as a highly specific marker for prostatic adenocarcinoma (17). Subsequent studies in Indian populations, including that by Rao et al. (2020), also reported higher AMACR intensity in high-grade lesions, supporting its role as a marker of tumor aggressiveness (18). However, AMACR expression in some benign mimickers such as atrophy and adenosis necessitates cautious interpretation, underscoring the importance of correlating IHC with morphology.

Ki-67, a nuclear protein expressed during cell proliferation, serves as a prognostic indicator in many malignancies. In the present study, the Ki-67 proliferation index showed a clear upward trend with increasing Gleason grade. Low-grade tumors demonstrated <5% positivity, while high-grade lesions exceeded 20%, indicating enhanced proliferative activity. Similar trends were reported by Mazzucchelli et al. (2016), who demonstrated that Ki-67 index correlates with Gleason score, tumor stage, and biochemical recurrence (19). Indian studies by Kakkar et al. (2021) and Kaur et al. (2019) have also highlighted Ki-67 as an independent prognostic factor for disease progression and metastasis (20,21). Thus, Ki-67 can complement histological grading by quantifying proliferative potential, aiding in risk stratification.

The tumor suppressor gene p53 plays a pivotal role in regulating cell cycle and apoptosis. Overexpression of p53 protein, usually due to gene mutation, is associated with genomic instability and aggressive behavior. In this study, p53 positivity was observed in 40% of cases, predominantly in higher-grade tumors, consistent with the association between p53 accumulation and dedifferentiation. Similar observations were made by Shah et al. (2020), who found strong p53 nuclear staining in high-grade and metastatic prostate carcinomas (22). Gupta et al. (2017) also reported a significant correlation between p53 positivity and high Gleason score in Indian patients (23). These findings suggest that p53 overexpression serves as a surrogate marker for molecular aggressiveness and may predict poor clinical outcomes.

When comparing results internationally, studies from Western populations demonstrate similar biomarker trends, but with variable expression thresholds. For instance, Bubendorf et al. (2018) reported that Ki-67 and p53 overexpression were significantly associated with biochemical recurrence and reduced survival (24). In contrast, Asian studies such as those by Takahashi et al. (2019) indicated slightly lower Ki-67 indices, possibly due to genetic and environmental differences (25). This variation highlights the importance of region-specific data to refine prognostic models.

Recommendations

1. **Routine IHC Panel:** Incorporating PSA, AMACR, Ki-67, and p53 into routine diagnostic panels can improve the accuracy of prostate adenocarcinoma diagnosis, especially in morphologically ambiguous cases.
2. **Prognostic Stratification:** Ki-67 and p53 should be utilized for prognostic evaluation and patient risk stratification in conjunction with Gleason grading.
3. **Integration with Molecular Profiling:** Combining IHC with emerging genomic markers may enhance personalized therapeutic strategies for prostate cancer management.

Limitations

1. **Sample Size:** The study was limited by a relatively small sample size, which may affect statistical generalization.
2. **Lack of Follow-up Data:** Absence of patient survival and recurrence data restricted assessment of prognostic outcomes.
3. **Technical Variability:** Variations in IHC staining and interpretation may introduce observer bias despite standardized protocols.

CONCLUSION

The present study demonstrates a significant correlation between histopathological grading and immunohistochemical markers in prostate adenocarcinoma. PSA expression decreased with increasing grade, reflecting loss of differentiation, while AMACR showed stronger positivity in higher grades. The Ki-67 proliferation index and p53 overexpression were notably higher in poorly differentiated tumors, indicating aggressive biological behavior. These findings emphasize that combining morphological assessment with IHC markers enhances diagnostic accuracy and prognostic evaluation.

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