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Advances in biomarker discovery and radiological techniques for early detection of ovarian cancer: A comprehensive review

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***Abstract*---**Aim: This review investigates recent advances in biomarker discovery and radiological techniques for the early detection of ovarian cancer, emphasizing the significance of early diagnosis in improving patient outcomes. Methods: A comprehensive literature review was conducted, focusing on various biomarkers, including CA125, HE4, and emerging candidates such as circulating tumor DNA and microRNA, alongside radiological imaging techniques such as ultrasound and MRI. The sensitivity and specificity of these biomarkers and imaging modalities were analyzed through clinical studies and trials. Results: Current biomarkers like CA125 and HE4 demonstrate varied sensitivities and specificities, with CA125 having low sensitivity in early stages but higher specificity. Radiological techniques provide crucial complementary information, enhancing diagnostic accuracy. Novel approaches, such as the Risk of Ovarian Cancer Algorithm (ROCA) and multivariate index assays like OVA1 and ROMA, show promise in enhancing diagnostic accuracy. Additionally, potential biomarkers, including glycoforms of CA125, autoantibodies, and methylation changes, have emerged as significant candidates for further research. Conclusion: While significant progress has been made in biomarker and radiological technique development for ovarian cancer, challenges persist in achieving the ideal sensitivity and specificity for early detection. Continued research and validation of novel biomarkers and imaging techniques are essential for developing effective screening methods, ultimately improving survival rates.

***Keywords*---**ovarian cancer, biomarkers, early detection, CA125, HE4, ROCA, multivariate assays, circulating tumor DNA, microRNA, radiological imaging.

Introduction

In the world, ovarian cancer is the eighth most common and the fifth most deadly type that affects women. The disease is a major threat to women's health and longevity, as evidenced by the fact that over 300,000 women are diagnosed with it each year and roughly 152,000 die from it, with an incidence rate of 3.4% and a fatality rate of 4.7% [1]. With only a 30% chance of survival, those with ovarian cancer have a dismal prognosis [2]. Platinum-based chemotherapy and cytoreductive surgery are currently used as first-line treatments [3]. For particular patient populations, targeted treatments such PARP inhibitors and anti-VEGF antibodies may be used [4]. However, more than half of the patients return within two years, leading to only modest improvements in survival rates

[5,6]. Studies reveal that the total five-year survival rate for early-stage diagnoses is almost 92%, which is significantly higher than the 29% rate for late-stage cases [7]. More than 70% of patients receive an advanced diagnosis because ovarian cancer progresses quickly from early to advanced stages in less than a year and lacks distinguishable early signs and symptoms [8]. As such, improving prognosis requires early detection and diagnosis [9].

Currently, a histological study is necessary for a conclusive diagnosis of ovarian cancer [10]. In order to remove the tumor, this usually requires surgery, which has associated operating risks. Furthermore, accurate preoperative assessment is crucial because the surgical techniques for benign and malignant ovarian tumors varies significantly. When it comes to ovarian cancers, transvaginal ultrasonography (TVS) is frequently the first method of detection. Even though a number of ultrasound characteristics have been found to signal cancer, more optimization is needed to ensure an accurate diagnosis [11]. Serum biomarkers provide a practical, affordable, and non-invasive option for predicting malignancy, and research is being done to find more trustworthy biomarkers for ovarian cancer early detection. The search for efficient screening techniques is still continuing. In order to acquire a positive predictive value (PPV) of at least 10%, a screening test for early-stage ovarian cancer must show a sensitivity surpassing 75% and a specificity of at least 99.6% [12]. The discussion that follows focuses on the latest developments in biomarker development for the early detection of ovarian cancer. These developments include the discovery of two FDA-approved biomarkers, pertinent indices or algorithms, and ongoing research into possible molecular biomarkers.

Routine screening for early detection has proven beneficial for various cancer types. For instance, since the implementation of the Papanicolaou (Pap) test, the incidence and mortality rates of cervical cancer in the U.S. screened populations have decreased by more than 75% [10]. Similarly, colonoscopy screening has been linked to a 70% reduction in mortality risk for colorectal cancer [11]. Unfortunately, for high-grade serous carcinoma (HGSC), effective screening methods are not yet available, as there is “currently no strategy for early detection screening that reduces mortality or incidence of ovarian cancer” [12]. Consequently, routine screening for HGSC is currently not endorsed by the United States Preventative Services Task Force (USPSTF) because “the potential harms outweigh the potential benefits” [12]. This decision is primarily influenced by the low prevalence of the disease in the general population, which would likely result in high rates of false positives from inadequate tests, potentially leading to unnecessary surgical interventions and psychological distress for women without ovarian cancer. Despite this challenge, the urgent clinical need for precise screening and diagnostic tests for ovarian cancer persists. Many clinicians and researchers are actively seeking innovative methods for early-stage disease identification from a variety of biomarker sources (**Figure 1**).

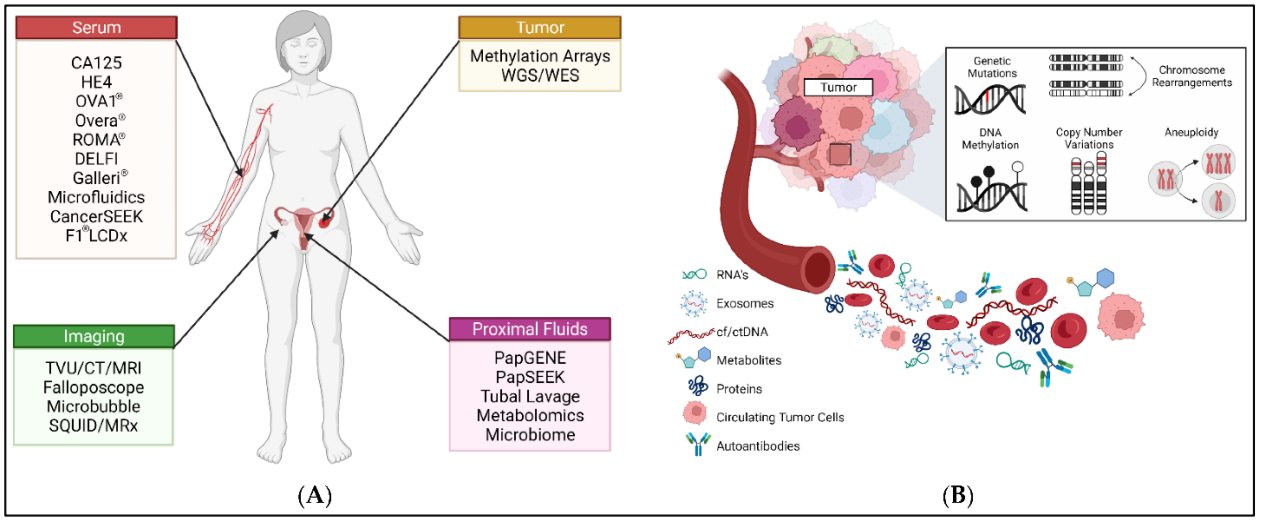

Figure 1: Ovarian Biomarkers

Current Ovarian Cancer Biomarkers:

CA125, or Cancer Antigen 125: The MUC16 gene produces the glycoprotein CA125, which is secreted into the bloodstream by coelomic and müllerian epithelia [13]. It can distinguish between malignant ovarian tumors and the general population since it is overexpressed in more than 80% of ovarian cancer cases [14]. For women displaying symptoms suggestive of ovarian cancer, the UK's National Institute for Health and Care Excellence (NICE) endorsed CA125 as a screening test in 2011 [15]. A CA125 level more than 35 U/mL suggests a higher risk of cancer in postmenopausal women. CA125 continues to be the most widely researched and widely used serum biomarker for the diagnosis of ovarian cancer. Except for CA125, no markers were found to be useful for detection when ovarian cancer was discovered more than nine months after blood work, according to a study by Mukama et al. that evaluated 92 preselected proteins in blood samples drawn less than 18 months before an ovarian cancer diagnosis [16].

Sensitivity and Specificity: Only over 50% of early-stage patients have high CA125 levels, which means that the sensitivity (50–62%) for early detection is low. As a result, the utility of CA125 in ovarian cancer early-stage detection is restricted. The ability to differentiate between advanced-stage patients and healthy controls was better with CA125 levels. In a retrospective investigation of CA125 levels prior to the diagnosis of ovarian cancer, Funston et al. discovered that patients with normal CA125 levels had a higher chance of receiving an early diagnosis than those with raised levels. As a result, depending just on CA125 for screening may cause women to experience worse results and a delay in diagnosis [17]. Furthermore, the specificity of CA125 is not very high (about 73–77%), and more than 60% of those with elevated CA125 do not have ovarian cancer [18]. Pregnancy, the menstrual cycle, various malignancies (including gastric, breast, uterine, pancreas, liver, and colon cancers), as well as benign illnesses such endometriosis, adenomyosis, acute pelvic inflammation, and uterine fibroids, can

all cause elevated CA125 [19]. 31.5% of Asian women with benign diseases in a study involving 414 women with adnexal masses had increased CA125 values [20]. The Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial results showed that the PPV for CA125 alone was only 3.7% [21]. This results in a decreased positive predictive value (PPV). The efficacy of CA125 varies according to the kind of ovarian cancer; non-epithelial malignancies, clear cell carcinomas, undifferentiated carcinomas, and mucinous carcinomas all perform poorly with this drug [22]. Diverse factors may also impact blood CA125 levels, resulting in variations in starting points amongst women.

Better Methods for Finding CA125: Several tactics have been used to improve CA125's diagnostic performance. There are now new methods available for determining serum CA125 levels. To measure serum CA125 levels, a double determinant immunoassay using an anti-MUC16 antibody (OC125) and an anti-IgM antibody (M11) is currently used. But there are drawbacks to this approach: these antibodies might not detect all repeats, and they might react with other proteins, which would lower the sensitivity and specificity [23, 24]. Novel detection techniques have been devised to solve these problems. An new antibody–lectin ELISA technique was developed by Wang et al. [25]; while it exhibited limited efficiency for borderline ovarian cancers, it revealed increased specificity for discriminating patients with positive CA125 levels. Furthermore, Schuster-Little et al. developed a mass spectrometry-based CA125 detection technique that revealed molecular areas that antibodies do not recognize [26]. Additionally, by immobilizing CA125 antibodies onto CuBTC@MoS2-gold nanoparticle (AuNP)-functionalized electrodes via electrostatic adsorption, nanoparticles can improve sensitivity and specificity of CA125 detection [27]. A viable method that has demonstrated better performance than conventional serum detection techniques is the detection of CA125 within exosomes, which produces better results in terms of area under the curve (AUC) (0.9755 vs. 0.9093), sensitivity (94.55% vs. 87.27%), and specificity (92.73% vs. 90.91%) [28].

Finding the CA125 Glycoforms: Finding the glycoforms of CA125 is another way to increase its effectiveness. Over two-thirds of the molecular weight of CA125 is attributable to glycans, including many N- and O-glycans on the extracellular amino terminal domain. This indicates that CA125 is extensively glycosylated [29]. Because of changed glycosylation processes during oncogenic transformation, tumor tissues may have shortened or abnormal carbohydrate side chains [30]. Ovarian cancer has been linked to aberrant N-glycosylation and shortened O-glycans of CA125, which may be used to distinguish patients from healthy people [25,31]. Compared to conventional serum protein assays, research has shown that the use of CA125 glycoforms can improve specificity and sensitivity for early ovarian cancer detection.

Thomsen-nouveau (Tn) antigens (Gal-NAc1-O-Ser/Thr), an O-glycan that is increased in ovarian cancer tissues but remains low in normal cells, are significantly present in CA125. In order to diagnose ovarian cancer, Wang et al. used an antibody–lectin (Vicia Villosa Lectin) ELISA assay to evaluate the combined levels of CA125 and Tn (CA125-Tn). With a fixed sensitivity of 90%, our approach demonstrated a much higher specificity (75.5% vs. 35.1%) in patients over 45 years of age, outperforming traditional CA125 immunoassays. This

method can also be used to detect CA125-Tn at low concentrations [25]. The sialyl-Tn antigen (STn), which is restricted in normal tissues but common in mucin-type glycoproteins across a range of human adenocarcinomas, is produced when sialylation of the Tn structure occurs. It has been shown that in patients with ovarian cancer, endometriosis, and healthy controls, the total serum STn antigen concentrations increased by 50%, 9.6%, and 3.8%, respectively. Numerous techniques have been developed to detect CA125-STn, such as glycovariant-based lateral flow immunoassays (LFIA) [33], time-resolved fluorometry immunoassays [32], and fluorescent europium nanoparticles coated with anti-STn monoclonal antibodies [34, 35]. Compared to conventional CA125 tests, these methods have continuously demonstrated superior performance, especially in terms of increasing sensitivity and decreasing false positives. Promising progress has been made in ovarian cancer early detection, with notable gains observed in postmenopausal cases and individuals with slightly elevated serum CA125 levels. However, the CA125-STn method is not as sensitive for detecting other ovarian cancer histologies, namely mucinous and clear cell tumors.

The ROCA algorithm (Risk of Ovarian Cancer): The extended biological half-life of CA125 implies that ongoing blood level monitoring could be advantageous [36]. Based on consecutive CA125 values, the Risk of Ovarian Cancer Algorithm (ROCA) assay evaluates the risk of ovarian cancer [37]. The goal of this assay is to monitor appreciable rises in CA125 levels so that those with noticeably elevated levels can be evaluated further using transvaginal ultrasonography (TVS) [38]. A single-threshold CA125 test's specificity limitations are improved when ROCA and TVS are combined, increasing sensitivity to 85% for earlier identification [39]. The mean trends (MMT) assay produced an area under the curve (AUC) of 0.911 and a sensitivity of 90.5% in a longitudinal trial with 360 postmenopausal women [37]. Annual TVS screenings as well as annual multimodal screenings (including longitudinal CA125 and second-line TVS) were investigated in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). In comparison to an unscreened group, this study showed that the CA125 screening led to a slight change in cancer stage, with a 47.2% increase in stage I cancer incidence and a 24.5% decrease in stage IV cancer incidence. After long-term follow-up (median follow-up > 16 years), this shift was found to be insufficient to produce a statistically significant reduction in mortality [40].

Secretory protein 4 of the human epididymis (HE4): Whey acidic four-disulfide core (WFDC) proteins, of which HE4 is a member, were first discovered in the distal epididymis epithelium [41]. It functions as a peptide protease inhibitor that is a part of the epithelial tissues' innate immune response [42, 43]. HE4 is overexpressed in ovarian cancer tissues, where it is released into the extracellular environment and found in the bloodstream, despite being missing in the ovarian surface epithelium [44]. Therefore, the identification of serum HE4 may serve as a possible biomarker for the detection and tracking of ovarian cancer.

Sensitivity and Specificity: Studies reveal that HE4 levels have a 96% specificity and 67% sensitivity in identifying ovarian cancer [45]. When it comes to benign gynecological disorders, HE4 is less affected than CA125; in individuals with adenomyosis, it is only slightly elevated and does not show elevation in

endometriosis [46]. Chan et al. discovered that whereas HE4 was not highly expressed in clear cell carcinomas, it showed increased sensitivity in mucinous tumors [20]. More than half of ovarian tumors that did not produce CA125 had high HE4 levels. It should be noted that HE4 expression is not limited to ovarian cancer; it is also highly expressed in mesotheliomas, lung adenocarcinomas, squamous cell carcinomas, breast adenocarcinomas, and endometrial cancer. The pooled sensitivity for HE4 for early ovarian cancer was determined to be 0.64, with a corresponding specificity of 0.87 [47]. The pooled sensitivity and specificity for late-stage ovarian cancer were 0.89 and 0.86, respectively [48]. In comparison to CA125, HE4 showed stronger specificity (96.9% vs. 67.1%) and PPV (78.7% vs. 35.8%), even though CA125 has been reported to have better sensitivity in late-stage patients (90.8% vs. 56.9%) [49]. In order to detect borderline or malignant ovarian tumors, a comprehensive review that included 49 research on the diagnostic role of HE4 and involved 12,631 women and 4,549 ovarian cancer patients found that the diagnostic tool had a pooled sensitivity of 0.78 and specificity of 0.86 [50].

Elements Influencing HE4 Amounts: Both age and menopausal state affect HE4 levels. Postmenopausal women have higher detection efficiencies, with specificities of 88% and 91%, respectively, and sensitivities of 77% versus 71% in premenopausal women [48]. Furthermore, HE4 levels tend to rise with age, which may cause older populations' specificity and sensitivity to decline [51]. Comparative research revealed that the sensitivity for HE4 detection was 100% in women under 50, but it dropped to 87.5% in women over 50. The older cohort also saw a decline in specificity, from 88.4% to 60.4% [50].

The Ovarian Cancer Multivariate Index Assays Available Currently

Assay for the Risk of Malignancy Index (RMI): Acknowledging the shortcomings of individual serum biomarkers, scientists have merged several indices to improve diagnostic efficacy. The Risk of cancer Index (RMI) was developed in 1990 by Jacobs et al. to estimate the risk of ovarian cancer (RMI = U × M × CA125) by integrating ultrasound results (U), menopausal status (M), and CA125 values [52]. Compared to CA125 alone, the RMI showed improved sensitivity (71–88%) and specificity (74–92%) with a cut-off value of 200 [53]. Later iterations, such as RMI 2, RMI 3, and RMI 4, were created with updated scoring for menopausal status and ultrasound. Tumor size is also taken into account by RMI 4, which achieves 86.8% sensitivity and 91% specificity [54,55,56]. While RMI 1 was the most accurate of the original versions, more recent research revealed that there were no appreciable variations in the area under the curve (AUC) across all variations [58]. RMI 1 is suggested by the NICE recommendations [57] for the management of suspected ovarian cancers.

Assay for OVA1: The risk index score for ovarian cancer is determined by combining serum biomarkers CA125, transthyretin, transferrin, beta-2 microglobulin, and apolipoprotein A-1 through the use of OVA1, an FDA-approved multivariate index test. With a sensitivity of 92% versus 79% and a negative predictive value (NPV) of 97% versus 93%, OVA1 has been demonstrated to perform better than CA125 alone [59]. OVA1 is especially useful for detecting

patients with ovarian cancer who are in the early stages of the disease as well as those with uncommon histological subtypes such mucinous and clear cell carcinomas that CA125 screening may miss [60,61,62].

The ROMA (Risk of Ovarian Malignancy) Assay: The ROMA, which was created by Moore et al. and received FDA approval for ovarian cancer diagnosis in 2010, integrates menopausal state, HE4, and CA125 into a logistic regression model [46,63]. ROMA's dependability for clinical diagnosis was highlighted by a meta-analysis of 5,954 cases, which showed a pooled sensitivity of 90%, specificity of 91%, and AUC of 0.96 [65]. Although postmenopausal individuals have a reduced specificity, ROMA exhibits better sensitivity in postmenopausal women when compared to premenopausal women [66]. It has been observed that the ROMA index improves predictive value, especially when CA125 or HE4 alone would not be sufficient [67,68]. By utilizing OVA1's sensitivity and ROMA's specificity in that order, sequential application of OVA1 and ROMA may maximize predictive results [69].

The ADNEX Model and the IOTA Basic Rules: Based on ultrasound examination, the International Ovarian Tumor Analysis (IOTA) basic guidelines show a 92% sensitivity and 96% specificity [70]. When evaluating pelvic masses, research has demonstrated that the IOTA basic guidelines are superior to the RMI or ROMA, especially in circumstances where results are unclear. Expert ultrasound also offers greater sensitivity than ROMA [70]. The Assessment of Different Neoplasias in the Iexa (ADNEX) model was also created by the IOTA group [71]. It uses clinical characteristics (age, serum CA125 levels, and type of center) in conjunction with ultrasound features to identify malignancy subtypes. The ADNEX model achieved a sensitivity of 86.5% at 90% specificity, surpassing previous models (such RMI and simple rules) in diagnosis accuracy, according to a research comprising 4,905 patients [72].

Potential Biomarkers for Ovarian Cancer Detection

Protein Biomarkers: Over 100 potential protein biomarkers have been studied for ovarian cancer detection. Folate receptor alpha (FOLR1) is notably overexpressed in high-grade ovarian cancer and can be detected in serum, showing increased specificity compared to CA125 [73-77]. CA72-4, another tumor-associated glycoprotein, has potential for detecting clear cell and mucinous carcinomas, where CA125 and HE4 may not be elevated [78-84]. Transthyretin (TTR) is downregulated in ovarian cancer and may be useful when combined with other biomarkers for early detection [85-86]. Other potential biomarkers include CA15-3, glycodelin, and kallikrein 11, but none surpass CA125 alone [78-87].

Multivariate Index Assays: Several multivariate index assays have been developed. The Copenhagen Index (CPH-I) uses age and serum levels of HE4 and CA125, achieving 69% sensitivity and 85% specificity [89-90]. The Risk of Ovarian Malignancy Index (ROMI) shows improved performance compared to ROMA [93]. Combined models incorporating multiple biomarkers have also demonstrated enhanced diagnostic capabilities, often exceeding CA125 alone in sensitivity and accuracy [94-97].

Autoantibodies (AABs): Autoantibodies generated in response to tumor-associated antigens (TAAs) can aid in cancer detection. The anti-TP53 autoantibody, present in about 20% of ovarian cancer patients, can be detected earlier than traditional markers like CA125 [100-101]. Optimized panels combining multiple AABs have improved sensitivity and specificity, showing promise for earlier diagnosis [102-107].

Circulating Tumor DNA (ctDNA): ctDNA, released from tumors, can be analyzed for genetic alterations associated with cancer. While promising for non-invasive detection, the sensitivity for early-stage ovarian cancer may be limited, particularly for small tumors [108-109]. Studies suggest ctDNA performs comparably to CA125 and HE4, but challenges remain regarding sensitivity and specificity [110-112].

Methylation Changes: Aberrant methylation of tumor suppressor genes occurs early in cancer development and can serve as a diagnostic marker. Methylation assays, especially for genes like HOXA9 and HIC1, have shown high sensitivity and specificity [113-116]. Methylation tests may outperform ctDNA assays in predicting ovarian cancer [110].

MicroRNA (miRNA): MiRNAs play a role in regulating gene expression and have shown differential expression patterns in ovarian cancer. Notable miRNAs include miR-200a-3p and miR-200c-3p, which show promising diagnostic potential [117-120]. Combining miRNAs with traditional biomarkers can enhance diagnostic accuracy, especially for early detection.

Main Role of Radiologist

Radiologists play a crucial role in the multidisciplinary approach to early ovarian cancer detection and management. Utilizing advanced imaging techniques such as ultrasound and MRI, radiologists provide essential insights into tumor characteristics, including location, size, and morphology. Their expertise in interpreting these images complements the data obtained from biomarker analyses, offering a comprehensive diagnostic picture. This collaborative effort between laboratory biomarker research and radiology ensures accurate diagnosis, effective treatment planning, and better patient outcomes, particularly in complex or atypical cases of ovarian cancer.

Conclusion

Ovarian cancer remains a formidable health challenge, characterized by late-stage diagnoses and poor prognoses. The search for effective biomarkers and radiological techniques has gained urgency, given the stark contrast in survival rates between early and late-stage detection. Current serum biomarkers like CA125 and HE4 have established roles in clinical practice; however, their limitations necessitate exploration of novel markers, diagnostic algorithms, and imaging modalities. CA125, although widely used, demonstrates low sensitivity for early-stage disease and is confounded by false positives from benign conditions. HE4, while showing improved specificity, also presents challenges,

particularly in specific tumor types. Emerging strategies, including the use of glycoforms, circulating tumor DNA, and novel autoantibody panels, offer promising avenues for enhancing diagnostic sensitivity and specificity. The integration of multiple biomarkers into multivariate index assays, such as OVA1 and ROMA, reflects a progressive shift toward more accurate and reliable screening protocols. These assays have shown superior performance compared to individual markers, particularly in identifying patients with early-stage or atypical histological subtypes of ovarian cancer. Furthermore, studies like the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) highlight the potential benefits of combining serum biomarker monitoring with imaging techniques to improve detection rates. Despite these advancements, the absence of universally endorsed screening strategies underscores the complexities inherent in ovarian cancer detection. In conclusion, while strides have been made in biomarker and radiological technique discovery for early ovarian cancer detection, continued research is imperative. The focus must remain on optimizing sensitivity and specificity, validating novel biomarkers and imaging techniques through robust clinical trials, and developing comprehensive screening protocols that can facilitate early intervention and ultimately improve patient outcomes.

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