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Enhancing diagnostic precision in thyroid nodule assessment: evaluating the efficacy of a novel cell preservation technique in fine-needle aspiration cytology

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Objectives: This study aimed to evaluate the effectiveness of thyroid fine needle aspiration cytology (FNAC) using a novel-cell preserving matrix called Cytomatrix in improving diagnostic accuracy for thyroid nodules.

Materials and methods: Fifty patients undergoing thyroidectomy were enrolled and FNAC was performed on the excised thyroid glands, with the collected sample being placed on the Cytomatrix. The results were compared with histopathological analysis, and diagnostic performance was assessed statistically.

Results: Cytomatrix demonstrated an accuracy of 96%, sensitivity of 84.61%, and specificity of 100%. Concordance between cytological and histopathological findings highlighted Cytomatrix's potential to enhance thyroid FNAC accuracy.

Conclusion: FNAC using Cytomatrix shows promise in improving diagnostic accuracy for thyroid nodules. Its application, marked by faster processing and efficient resource utilization, coupled with the preservation of cellular architecture, holds considerable potential in enhancing cytological diagnosis, thus optimizing patient management strategies.

1 Introduction

Thyroid nodular disease has become increasingly prevalent in clinical practice, owing to advancements in diagnostic methodologies, with a substantial majority of these nodules presenting as benign lesions (1). The continuous rise of environmental pollution (2, 3), dietary changes, radiation exposure, the use of certain medications, combined with individual genetic predisposition, represent the main contributors in the development of thyroid nodules, especially thyroid carcinoma (4–6). With advancing age, the incidence of thyroid nodules increases, showing prevalence rates ranging from 7% to 67%, and with a higher likelihood of occurrence in women (7). Thyroid nodules can be detected via self-palpation, though this depends on lesion size. More commonly, they are found incidentally during routine examinations, particularly through neck ultrasound (8). Recent years have witnessed a surge in the identification of smaller lesions, including thyroid carcinomas, which represent approximately 7-15% of all nodules (9). This rise is mainly due to advances in thyroid ultrasound technology, including elastography and contrast-enhanced ultrasound (CEUS) for assessing high-risk nodules (10–12).

Despite progress in imaging modalities for detecting suspicious thyroid lesions, distinguishing between malignant and benign nodules is a persistent challenge (13).

Fine needle aspiration cytology (FNAC) remains the gold standard in preoperative assessment of thyroid nodules, with high sensitivity and accuracy in differentiating malignant lesions, as presented in published literature, with sensitivity and specificity ratios ranging from 65% to 98% and 73% to 100%, respectively (14). FNAC of the thyroid is typically performed by specialized practitioners—endocrinologists, radiologists, or surgeons—who have undergone rigorous training (15, 16).

Experts use ultrasound guidance to precisely locate thyroid nodules. A 22-to-27-gauge needle extracts cellular material, which is then smeared on slides for cytological examination (17). Results are analyzed using the 2023 Bethesda System for Reporting Thyroid Cytopathology, which categorizes them into six groups: (i) nondiagnostic; (ii) benign; (iii) atypia of undetermined significance; (iv) follicular neoplasm; (v) suspicious for malignancy (SFM); and (vi) malignant (18).

Despite the utility of FNAC, a considerable proportion of samples yield inconclusive results, primarily due to inadequate material collection and processing, potentially resulting in interpretation errors and diagnostic uncertainty (19, 20). Immunohistochemical and molecular tests are limited with conventional smears due to cell architecture alterations. On the other hand, cell block preparation preserves cellular structure and improves diagnostic accuracy but is resource-demanding and time-consuming, leading to delays in diagnosis and management (21). It also requires more cellular material, with potential loss during preparation (22, 23).

The Cytomatrix (developed by UCS Diagnostic Srl & Campus Bio-Medico University of Rome, Rome, Italy) represents a cutting-edge advancement in cell preservation techniques tailored specifically for capturing and preserving the architecture of thyroid cells obtained after FNAC within its 3D synthetic matrix (24). This unique feature enables the obtained cytology to closely mimic the patterns observed in histological sections, requiring minimal intervention from the technician post-sample collection, and thereby substantially enhancing the accuracy of diagnosis.

Moreover, the preserved matrix can be stored indefinitely, offering the flexibility to perform additional diagnostic tests such as immunohistochemistry (IHC), fluorescence *in situ* hybridization (FISH), and molecular biology assays. Additionally, it presents the advantage of requiring a smaller volume of cell aspirate compared to traditional smears or cell blocks (24). This not only streamlines the sample collection process but also preserves valuable cellular material for further analyses, ultimately contributing to more comprehensive diagnostic evaluations.

The primary objective of this study was to assess the capability of the novel Cytomatrix in delivering accurate and precise diagnosis following FNAC of the suspicious thyroid nodules, by comparing the Cytomatrix cytological findings with the histopathological results of the excised thyroid glands, aiming to minimize the occurrence of inconclusive results or misdiagnoses, commonly associated with traditional cytology smears. Emphasis was placed on prioritizing an approach that is resource-efficient and time-effective.

2 Materials and methods

This prospective investigation centered on patients admitted to the First Surgical Department of Timisoara Emergency County Hospital, between March 2023 and March 2024, specifically those scheduled for thyroidectomy. Selection for surgery was based on suspicious ultrasound findings, the presence of compressive symptoms, and additional factors such as elevated calcitonin levels or a clinical diagnosis of Graves' disease in patients who did not respond to antithyroid medication. Fine needle aspiration cytology (FNAC) was performed on the excised thyroid gland of each patient enrolled. The selection criteria for performing FNAC on the specimens was established based on preoperative ultrasound evaluations, characterized by at least one suspicious nodule scoring 4 or higher on the European Thyroid Imaging and Reporting Data System (EU-TIRADS) (25). Preoperative FNAC was not conducted. Subsequently, fifty patients meeting these criteria were enrolled.

Adhering to the principles outlined in the Declaration of Helsinki, informed consent was obtained from all participants.

Following the surgical excision of the thyroid gland, the surgeon promptly conducted fine needle aspiration on the thyroid's most suspicious nodule, as determined by the preoperative ultrasound examination, prior to its immersion in formalin for subsequent histopathological analysis. The excised thyroid was placed on a sterile surface. Subsequently, the surgeon located the preoperative ultrasound-identified suspicious nodule through palpation. A 23-gauge needle attached to a 10ml syringe was used for sample collection (as shown in Figure 1). Combined capillary and aspiration techniques were used.

After the fine needle aspiration process, the collected sample (1-2 drops) was placed onto the Cytomatrix sponge (as shown in Figure 2) and immersed in 30ml formalin for at least 12 hours.

After fixation in formalin, the Cytomatrix underwent permanent embedding in paraffin and was subsequently sectioned using a
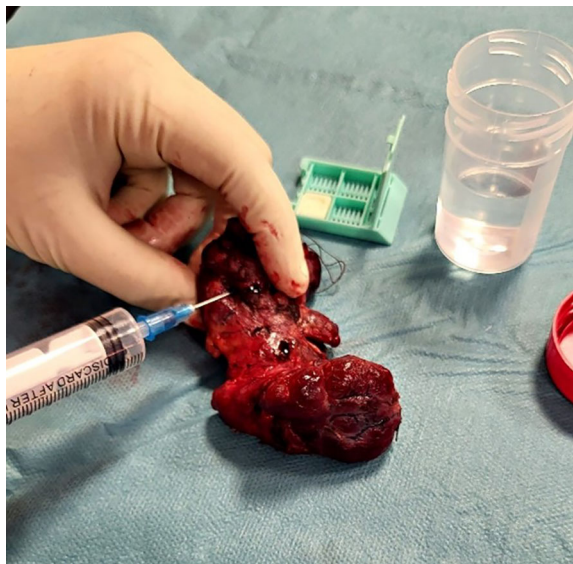
FIGURE 1
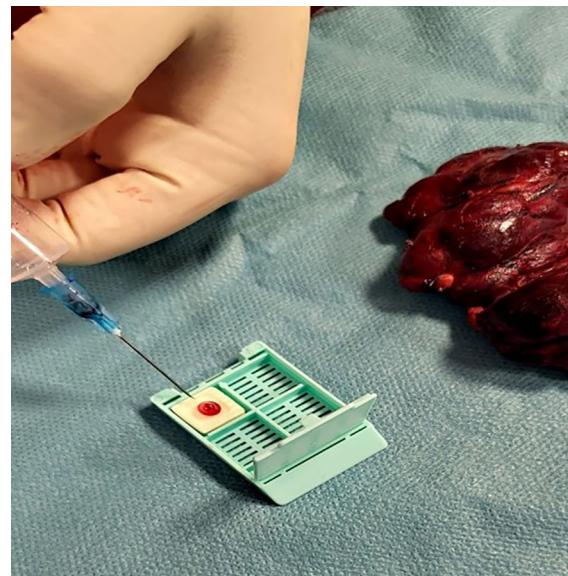FNA technique on the excised thyroid gland.

FIGURE 2
Placement of obtained material onto the Cytomatrix.

microtome to generate multiple 10-micron slices, which were then subjected to standard histological hematoxylin and eosin (HE) staining procedures. The excised thyroid gland underwent histopathological examination at the pathology department of the hospital using standard diagnostic protocols. The results were interpreted in accordance with the 2022 World Health Organization (WHO) histologic classification of thyroid neoplasms (26). To maintain a blind approach, the cytological analysis of the Cytomatrix sections was performed by a different pathologist than the one who conducted the histopathological examination of the excised specimens. Furthermore, the histopathological evaluation was carried out at a separate center to ensure unbiased results.

Because of the non-standardized nature of Cytomatrix, the cytological results were stratified into categories mirroring those of the 2023 Bethesda System, encompassing benign, equivocal, suspicious for malignancy, and malignant. This categorization was employed to establish a comprehensive diagnostic framework consistent with the principles of the 2023 Bethesda System. Cases in which acellular samples were acquired during FNAC, resulting in the production of non-diagnostic Cytomatrix outcomes akin to the Bethesda I category, were excluded from the analysis.

Ultimately, the cytology results were compared with the histopathological findings.

Fifty patients were included in the study, comprising individuals who underwent both histopathological and cytological examinations. The demographic characteristics, including age and sex of each participant, were recorded.

Cytological assessments were conducted on the Cytomatrix block. The results were categorized according to the classification scheme designed to parallel the 2023 Bethesda System.

Histopathological examinations were conducted on the excised thyroid gland in accordance with the 2022 World Health Organization (WHO) histologic classification of thyroid neoplasms, to confirm the presence of malignant or benign lesions. The number of patients where malignancy was present was recorded, along with the specific subtypes identified, and the number of patients with benign lesions, along with their subtypes were also documented.

The comparison between the Cytomatrix cytology results and histopathological findings are illustrated through descriptive statistics, including graphical representations. These representations encompass the prevalence of identified malignancies and their subtypes, benign lesions, and their subtypes, as well as demographic variables within the cohort of fifty patients. Statistical analysis was carried out using MedCalc Statistical Software version 20.111 (MedCalc Software Ltd., Ostend, Belgium).

3 Results

Fifty patients underwent total thyroidectomy in this study, with ages ranging from 21 to 74 years old. 7 were male and 43 were female.

Only nodules with a minimum preoperative ultrasonography (US) risk assessment of 4 (according to the US EU-TIRADS classification) were subjected to FNAC and were distributed as follows: 40 nodules classified as TIRADS 4 and 10 nodules classified as TIRADS 5.

In the cohort of fifty patients analyzed using the Cytomatrix methodology, the results were classified according to the classification employed to mirror the Bethesda system. The analysis yielded 37 cases classified in the benign category, 2 as equivocal, 9 as suspicious for malignancy, and 2 as malignant.

The Cytomatrix cytological results were validated in accordance with the final histological examination that uncovered 13 malignancies within the fifty-patient cohort. This comprised 9 instances of papillary carcinoma and 2 instances of follicular variant of papillary carcinoma, each in alignment with its

corresponding Cytomatrix diagnosis falling under the suspicious for malignancy or malignant category. The two cases initially categorized as equivocal risk on the Cytomatrix were subsequently identified as medullary carcinoma and follicular variant of papillary carcinoma upon histopathological examination.

Furthermore, the final histological examination classified the remaining 37 cases as benign lesions, mirroring the results of the Cytomatrix cytological analysis, which had categorized each of the corresponding 37 cases into the benign category. Their distribution was as follows: 28 cases of benign nodular goiter, 7 cases of chronic thyroiditis, and 2 cases of Graves disease. Although these two cases were initially diagnosed clinically preoperatively, both Cytomatrix and histopathological examinations detected microscopic features often associated with Graves' disease, such as hyperplasia and hypertrophy of the follicular cells, as well as lymphocytic infiltration. These findings further reinforced the diagnosis.

Out of the total fifty patients, Cytomatrix identified 11 malignancies (True Positives) 37 benign lesions (True Negatives), and 2 equivocal lesions (False Negatives). Histopathological examination identified 13 malignancies, and 37 benign lesions.

After the statistical analysis of the obtained data, the Cytomatrix cytological analysis exhibited an accuracy of 96%. Additionally, it revealed a sensitivity of 84.61% and a specificity of 100%. Furthermore, the positive predictive value (PPV) of the Cytomatrix was determined to be 100%. The two cases categorized as equivocal by the Cytomatrix were labeled as false negatives, contributing to a discordance rate of 15.39% between Cytomatrix and the histopathological exam.
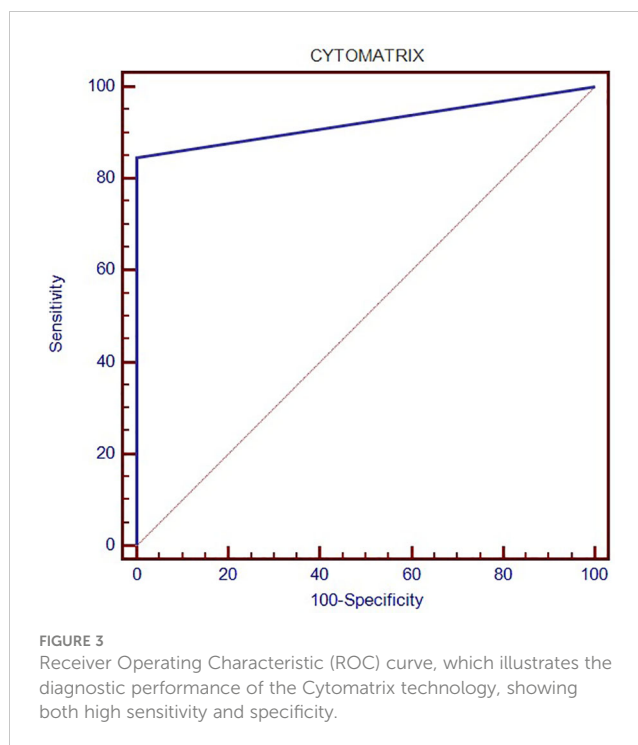
A graphical representation of the Cytomatrix performance is shown in Figure 3.

Obtained results and demographic data are represented in Table 1 (Abbreviations: SFM – Suspicious For Malignancy). Microscopic images depicting various instances of Cytomatrix findings from the fifty-patient cohort are displayed in Figure 4 showcasing papillary carcinoma with 40x magnification, Figure 5 showcasing papillary carcinoma with 20x magnification, and Figures 6, 7 showcasing benign nodular goiter with 10x magnification.

4 Discussions

The objective of this investigation was to evaluate the effectiveness of a novel cell-preserving matrix, known as Cytomatrix, in thyroid FNAC. By conducting a comparative assessment of the cytological outcomes obtained through Cytomatrix with the subsequent histological findings from the excised thyroid specimens of the same cohort of fifty patients, a valid concordance between the two diagnostic methods was established.

Cytomatrix exhibited encouraging outcomes, with a sensitivity of 84.61% and a specificity of 100%, reflecting its enhanced capacity to detect malignant cases among all true positives and its capacity in distinguishing benign lesions among the 37 true negative cases, respectively. Moreover, the positive predictive value (PPV) of Cytomatrix was established at 100%, underscoring its reliability in forecasting malignancies among the cases identified as true positives. The accuracy of 96% is comparable to values found in

FIGURE 3
Receiver Operating Characteristic (ROC) curve, which illustrates the diagnostic performance of the Cytomatrix technology, showing both high sensitivity and specificity.

published data regarding the accuracy of conventional smears in FNAC of the thyroid (27), further reinforcing the potential and efficacy of Cytomatrix in thyroid FNAC, providing a comprehensive evaluation of its diagnostic potential.

These findings show promise, especially when compared with published results obtained through conventional smear techniques. While certain studies have indicated lower sensitivity, specificity, and PPV with conventional smears in thyroid FNAC (28), others have reported outcomes like those observed in this study (29). Nonetheless, these findings emphasize the potential of Cytomatrix as a valuable tool in thyroid fine-needle aspiration cytology (FNAC).Two cases of equivocal lesions (equivalent to the Bethesda III category of indeterminate lesions) were identified on the Cytomatrix from the cohort of fifty patients, both of which were subsequently confirmed as malignant upon histological examination. This observation is consistent with existing literature suggesting a higher likelihood of malignancy confirmation upon histological evaluation for lesions categorized as Bethesda III (30). As only two cases in this study were categorized as equivocal lesions (equivalent to Bethesda III indeterminate lesions), it raises questions regarding the potential efficacy of Cytomatrix in reducing the occurrence of indeterminate lesions, particularly within the Bethesda III category. It prompts an inquiry into whether the use of conventional smears would have yielded more cases of indeterminate lesions (Bethesda III) within the fifty-patient cohort, or if the use of Cytomatrix genuinely contributed to minimizing such outcomes. Indeterminate lesions, specifically those falling into the Bethesda III category, are well-known for presenting a diagnostic challenge (31). However, Cytomatrix offers the possibility to perform additional ancillary tests, thereby enhancing diagnostic accuracy (24, 32).

Traditional smears remain a reliable, cost-effective, and straightforward method in thyroid (FNAC). Nonetheless, a

TABLE 1   Representation of the concordance between the Cytomatrix cytological results and histopathological results among the fifty-patient cohort.

Row Labels	Instances
BETHESDA II equivalent - BENIGN	**37**
Chronic Thyroiditis	**7**
Female	7
Benign Nodular Goiter	**28**
Male	5
Female	23
Graves Disease	**2**
Female	2
BETHESDA III equivalent - EQUIVOCAL	**2**
Medullary Carcinoma	**1**
Female	1
Follicular Variant of Papillary Carcinoma	**1**
Female	1
BETHESDA V equivalent - SFM	**9**
Papillary Carcinoma	**7**
Male	1
Female	6
Follicular Variant of Papillary Carcinoma	**2**
Male	1
Female	1
BETHESDA VI equivalent - MALIGNANT	**2**
Papillary Carcinoma	**2**
Female	2
Grand Total	**50**

SFM, Suspicious for Malignancy.
The words in bold represent the Bethesda categories and specific diagnoses within each category. These are highlighted to distinguish the classification and diagnosis of thyroid lesions, making it easier to differentiate between the various diagnostic outcomes.

notable proportion of cases analyzed using traditional smears yield inconclusive results or misdiagnoses, largely attributed to operator-dependent factors such as inadequate or insufficient material collection during the FNAC procedure (33, 34). This underscores the necessity for repeated FNAC or multiple passes to obtain an adequate sample, resulting in a time-consuming, cost-ineffective, and burdensome process for the patient (35, 36). In contrast, Cytomatrix requires a smaller volume of sampled material due to its distinctive 3D cell-preserving structure. With Cytomatrix, cells retain their original architecture instead of being smeared, allowing for a more efficient use of the sampled material during analysis. In each of the fifty cases presented in this study, 1-2 drops of FNAC sample placed on the Cytomatrix sponge, along with a single needle pass, proved adequate for achieving a reliable cytological diagnosis.

Both capillary and aspirate techniques were used in the material collection process during fine needle aspiration. The capillary method, regarded as a non-aspiration technique, is employed for its ability to minimize hemorrhagic material collection, as blood can often compromise sample adequacy. Although fine needle capillary sampling (FNCS) yields higher-quality material, fine needle aspiration cytology (FNAC) offers superior quantitative sampling. Each technique presents distinct advantages and limitations. A hybrid approach, incorporating both methods might yield superior fine needle cytology samples and enhance diagnostic accuracy (37, 38).

As indicated, non-diagnostic (Bethesda I equivalent) samples were omitted from the scope of this study.

Similar to conventional smears, Cytomatrix also carries the potential for generating non-diagnostic or inconclusive outcomes, largely due to insufficient cellular material. However, this risk is notably reduced by its distinctive capability to preserve collected material more effectively (24). Consequently, even with a lower quantity of cells, there is a higher likelihood of maintaining the unaltered quality of the sample. This characteristic distinguishes it from traditional smears, where the smearing process, along with subsequent fixation and staining techniques, poses a heightened risk of artifacts, cell overlap, morphological alterations of the sampled material, and potential cell loss (39, 40).

Another well-established method in thyroid cytology is represented by the use of cell blocks.

This technique has demonstrated its efficacy in diagnosis, primarily attributed to the superior preservation of cell architecture within the block, the same advantage Cytomatrix offers. Furthermore, the versatility of cell blocks extends beyond traditional cytological analysis offered by conventional smears, allowing for additional optimized ancillary tests such as molecular analysis, immunohistochemistry (ICH) or FISH (Fluorescence *In Situ* Hybridization). This multifaceted approach enhances the diagnostic capabilities, providing a more intricate analysis of the sampled material (41, 42).

The cell block technique encompasses various preparation methods, such as thromboplastin-plasma clot method, fibrin clot method, or cell blocks from liquid-based cytology. Each of these methods presents its unique approach (21, 43–45).

However, the creation of a single cell block demands additional cellular aspirate, as some of the collected material may be lost, depending on the type of processing technique (22, 46).

Therefore, it's noteworthy that regardless of the specific cell block technique employed, most of these methods share a common trait — they tend to be time-consuming and necessitate additional specialized equipment for optimal execution, and sometimes offer no added benefit to the final interpretation, resulting in delays in diagnosis and patient management (21, 47).

Cytomatrix, however, effectively addressed these limitations in this study, as there was no longer need for additional specialized equipment. Its distinctive design enabled the operator to directly deposit the collected material from FNAC of the thyroid, typically comprising 1-2 drops, onto the preserving sponge of the Cytomatrix complex, eliminating the requirement for additional processing. Following immersion in formalin, the Cytomatrix was embedded in paraffin and cut using a microtome, thus greatly simplifying the procedure for the final analysis.

One of the limitations encountered was insufficient cells on the matrix. These cases, as presented, were excluded from the study.
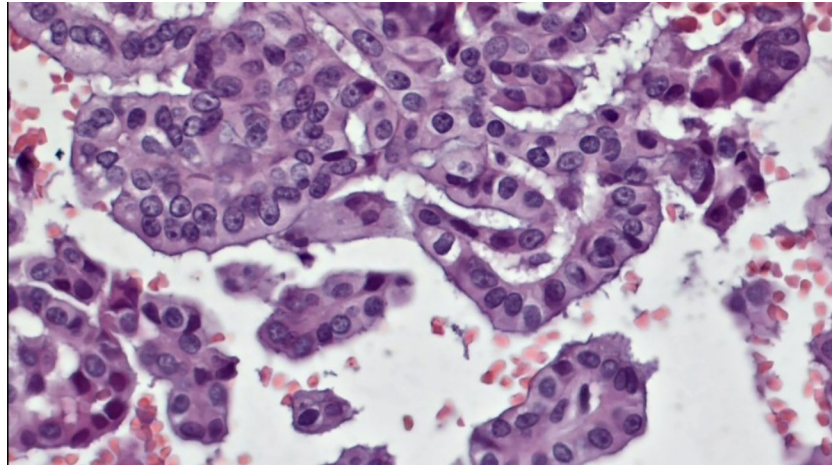
FIGURE 4
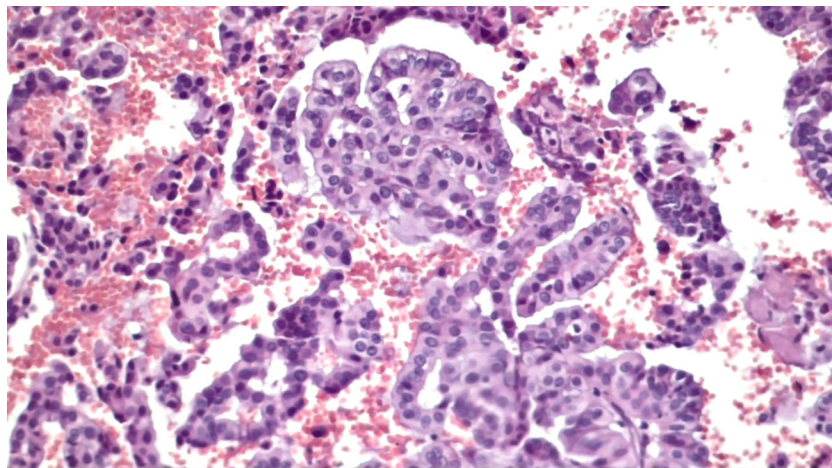Slides cut from the paraffin-embedded Cytomatrix showcasing papillary carcinoma – magnification 40x.

FIGURE 5
Slides cut from the paraffin-embedded Cytomatrix showcasing papillary carcinoma – magnification 20x.
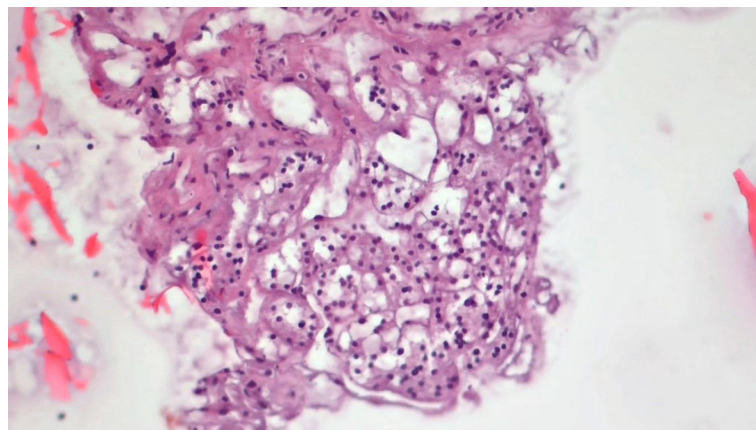

FIGURE 6
Slides cut from the paraffin-embedded Cytomatrix showcasing benign nodular disease – magnification 10x.
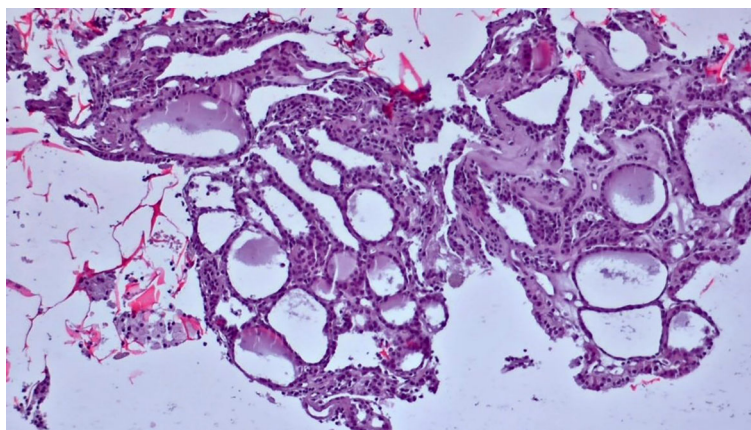
FIGURE 7
Slides cut from the paraffin-embedded Cytomatrix showcasing benign nodular disease – magnification 10x.

However, it is important to emphasize that this limitation solely stemmed from the operator's sampling technique. There were no instances of cell loss or alterations attributable to the Cytomatrix itself or its processing technique, as observed in the processing of conventional smears or various cell block preparation methods (22, 39).

Another advantage of the Cytomatrix is its capacity to generate multiple sections from the preserved paraffin block, facilitating long-term storage and future analyses. This sets it apart from conventional cytological smears, which are generally limited to a single use (25, 48). The Cytomatrix blocks obtained from the fifty-patient lot can be further used to perform additional ancillary tests, such as IHC, FISH and molecular biology, that are not otherwise optimized to be performed on conventional smears.

The integration of ancillary tests in thyroid cytology enhances diagnostic precision, enables personalized treatment approaches, and provides valuable insights into the molecular characteristics of thyroid lesions. These advanced techniques contribute to a more nuanced understanding of thyroid pathology, particularly in navigating the diagnostic complexities associated with well-known indeterminate lesions (Bethesda III), assisting clinicians in making informed decisions regarding patient management (49, 50).

In the current study, incorporating ancillary tests was deliberately avoided, emphasizing the commitment to assess the potential of Cytomatrix in thyroid FNAC while minimizing resource utilization. The assessment of its diagnostic efficacy, compared to conventional smears, relied solely on routine cytological analysis. This strategic approach aimed to emphasize the efficiency and cost-effectiveness of the Cytomatrix technique, even in the absence of supplementary ancillary testing. It underscores the potential of Cytomatrix in achieving precise diagnoses with minimal resource requirements, showcasing its effectiveness in routine practice.

Nevertheless, it is important to highlight that some studies opted to incorporate ancillary test in their evaluation using Cytomatrix, resulting in successful outcomes (51, 52). This highlights the versatility and effectiveness of the Cytomatrix, particularly when researchers choose to explore its full potential through these additional analyses.

The pathologist's perspective further showcases the efficacy of the Cytomatrix. When presented with matrices containing adequate material for analysis, the pathologist remarked that the experience was akin to examining a histology slide rather than traditional cytology. This observation not only emphasizes the matrix's effectiveness but also underscores its potential to elevate the diagnostic process to a level reminiscent of histopathological assessments.

The cytological characteristics of thyroid lesions play a crucial role in diagnostic accuracy and subsequent patient management. To evaluate the effectiveness of different cytological diagnostic methods in thyroid FNAC, a comparative analysis of Cytomatrix, conventional smears, and histological analysis was conducted. Table 2 presents a comprehensive comparison of these methods based on various parameters such as cellular architecture, nuclear traits, presence of artifacts, and ancillary testing capabilities (24, 53, 54). While these traits are primarily based on established literature, some of them are reinforced by the findings from this study. It is meant to highlight that Cytomatrix has the potential to be a significant advancement in thyroid fine-needle aspiration cytology (FNAC) compared to conventional smears. The close resemblance between Cytomatrix cytological analysis and the histological examination underscores its role in bridging the gap between cytology and histology. This positions Cytomatrix as a promising advancement in thyroid FNAC, offering the potential for enhanced diagnostic accuracy and supporting more precise patient management strategies.

One significant limitation of this study is that FNAC was performed on excised thyroid glands, rather than preoperatively, directly on the patients. This approach was chosen because the study is a preliminary evaluation of the relatively new Cytomatrix technology to determine whether it can be used for cytological evaluation of thyroid lesions with minimal processing after FNAC sample collection. Another limitation encountered was the inadequacy of the sampled material, specifically the insufficient number of cells on certain Cytomatrixes, which led to their exclusion from the analysis. Future research will try to address these limitations by performing preoperative FNAC on patients *in vivo*, using Cytomatrix. The insights gained from this study will help refine the technology and validate its effectiveness for direct clinical application.

TABLE 2 Comparison between Cytomatrix, conventional smears and histopathological exam in thyroid FNAC.

Exam Type / Traits	Cytomatrix	Conventional Smears	Histopathological Exam
Cellular Architecture	**Preserved** Well-preserved cellular arrangements (follicles, micro follicles, papillae)	**Limited** Poorly preserved cellular arrangements (follicles, micro follicles, and papillae)	**Preserved** Well-preserved cellular arrangements
Nuclear Characteristics	**Preserved** view of clearings, grooves, pseudo inclusions, and overlapping	**Limited** view of clearings, grooves, pseudo inclusions, and overlapping	**Preserved** view of clearings, grooves, pseudo inclusions, and overlapping
Artifacts	**Limited**	**Common** (fibrin inclusion/obscuring blood due to smearing process)	**Limited**
Ancillary Testing	**Suitable**	**Limited**	**Suitable**

The bolded terms "Cellular Architecture," "Nuclear Characteristics," "Artifacts," and "Ancillary Testing" represent specific traits being evaluated across the three different exam types. The words "Preserved," "Limited," and "Suitable" are also bolded to highlight how each trait is assessed for each exam type.

5 Conclusions

This study shows that Cytomatrix is a promising advancement in thyroid FNAC, effectively preserving cell architecture and offering high diagnostic accuracy. With a sensitivity of 84.61% and a specificity of 100%, Cytomatrix proved reliable in identifying malignant lesions, showing a potential improvement over conventional smears. The positive predictive value of 100% further supports its efficacy in detecting malignancies. Although the Cytomatrix identified two cases as equivocal, which were later confirmed as malignant, its overall performance aligns well with histopathological results, showcasing an accuracy of 96%.

The study highlights Cytomatrix's capability to maintain cellular architecture and support additional testing, with minimal sample material required. This approach not only streamlines the diagnostic process but has the potential to reduce the need for repeated FNACs. The integration of Cytomatrix into routine practice could significantly enhance diagnostic precision, reduce resource use, and improve patient management by minimizing inconclusive results. Future research will focus on validating Cytomatrix in preoperative, *in vivo* FNAC scenarios to further refine its clinical application and address the limitations observed in this pilot study.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by The Ethics Committee for Scientific Research of the "Pius Brinzeu" Emergency County Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

D-RS: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. ON: Writing – review & editing, Software, Investigation. AB: Writing – review & editing. II: Writing – review & editing, Software, Investigation. MD: Writing – review & editing, Visualization, Investigation. DS: Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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