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Accuracy of Intraoperative Frozen Section Diagnosis: A Three-Years Study at Dr. Mohammad Hoesin Central General Hospital, Palembang

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ABSTRACT

Background
A frozen section or vries coupe (VC) is an examination procedure performed by a pathologist during a patient operation on the surgical table. VC provides immediate information to surgeons regarding the benign or malignant status of a tissue, as well as determines the incision margin free from tumor mass.

Objective
This study aimed to evaluate diagnostic accuracy using the slide preparation archives of VC and histopathology at the Anatomic Pathology Department of Dr. Mohammad Hoesin Central General Hospital (RSUP), Palembang.

Methods
Samples of patients were collected from 1 January 2021 to 1 October 2023, while sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for VC were assessed compared to the definitive diagnosis of histopathology. A reassessment was conducted by two pathologists on slide preparations with unmatched results between VC and histopathological diagnosis.

Result
Additionally, VC examination showed sensitivity, specificity, PPV, and NPV of 99.7%, 99.7%, 98.3%, and 98.3% for benign tumors as well as 98%, 99.6%, 98.3%, and 98.3% for malignant tumors, respectively. The reassessment of the inappropriate slide preparations obtained a weighted kappa value of 0.586, signifying moderate agreement. Diagnostic discordance is caused by the experience of pathologists, freezing artifacts, and challenges in obtaining precise samples under a limited timeframe.

Conclusion
In conclusion, this study provided valuable insights into the effectiveness of VC procedure in intraoperative tissue diagnosis, showing the need for collaborative efforts and experienced pathologists to achieve improved accuracy.

Keywords: accurate, frozen section, histopathology, intraoperative

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INTRODUCTION

A frozen section (FS) or vries coupe (VC) is an Anatomic Pathology (PA) examination performed to rapidly diagnose lesions during a patient operation on the surgical table. Large hospitals are often equipped with cryostatic machines that can freeze tissue and create preparations for VC, enabling rapid intraoperative diagnosis.[1] Fresh tumor tissue without formalin liquid fixation is used for VC, a method comprising the dissection and examination of tumors or cancer cells during surgery to obtain immediate results, facilitating the determination of subsequent operation type needed. Implementing this unique and valuable method streamlines patient care through the prompt identification of benign or malignant lumps by pathologists to ensure appropriate treatment. Additionally, frozen slices or cuts are used for immunofluorescence and enzyme immunochemistry tests, as well as to determine tissue fat and carbs composition.[2]

VC analysis is crucial in determining the neoplastic or non-neoplastic nature of lesions, where those found to be neoplastic are further identified as benign or malignant. This method includes the rapid freezing of a small sample of tissue presented with lesions, followed by a microscopic examination for any cellular abnormalities. The frozen tissue is cut into thin slices, which are stained with special dyes to enhance the visibility of cellular structures. Subsequently, the slides are examined under a microscope to identify the presence of abnormalities, such as aberrant cell morphology or mitotic figures. This process is relatively quick, lasting only 30 minutes, and can be performed while the patient is under anesthesia on the surgical table.[3,4]

Pathologists face a significant challenge during VC analysis, suggesting the necessity for collaboration to ensure accurate diagnosis along with the provision of suitable assistance to physicians and surgeons while implementing this process. By leveraging joint expertise and experience, pathologists can provide precise diagnosis and consultation responses, enabling informed decisions about patient care. Therefore, effective teamwork is crucial to overcoming the complexities of intraoperative consultation or VC and achieving the best results for patients.[4]

VC [illegible] a useful tool in [illegible]s during surgery, but [illegible]tations compared to histopathological [illegible]nation with paraffin blocks. These limitations include sampling errors and insufficiency, as well as technical challenges. The need to reach prompt decisions under pressure and in a short timeframe during VC examination signifies the relevance of the experience and knowledge of anatomical pathologists in making an accurate diagnosis.[5]

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METHODS

This observational study with a retrospective cross-sectional design was conducted from 2021 to 2023, while both primary and secondary VC data were collected using the consecutive sampling method. The secondary data comprising sociodemographic characteristics, clinical history, as well as patient VC and histopathology diagnostic results, were obtained from the medical records of the Anatomic Pathology Department of Dr. Mohammad Hoesin RSUP, Palembang.

Collected tissue samples were assessed for positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity. In cases of frozen samples providing different results between VC and histopathological diagnosis, two medical professionals in the Anatomic Histopathology Department conducted a re-examination. Additionally, interobserver agreement was evaluated using Cohen-weighted κ, and data analysis was performed with IBM SPSS 25. Ethical issues were thoroughly addressed in accordance with relevant guidelines and regulations, while the study protocol was approved by the Ethics Committee ("Ethical Exemption" ID. DP.04.03/D.XVIII.6.11/ETIK/66/2023).

RESULT

Demographic data

VC examination included identifying the demographic characteristics of 577 patients across a three-years period, ranging from 2021 to 2023. Moreover, the dataset obtained constituted the complete cases recorded at the Anatomic Pathology Department of Dr. Mohammad Hoesin RSUP, Palembang.

A total of 577 patients were subjected to VC intraoperative diagnostic examination in the Anatomic Pathology Department. VC cases recorded in 2021, 2022, and 2023 amounted to 168, 237, and 172, respectively, comprising 7.3% male and 92.7% female (Figure 1).

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Table 1. Demographic data of patients subjected to VC examination between 2021-2023.

Parameter	Years			*n* (%)
	2021	2022	2023	
Gender				
Male	6	16	20	42 (7.3)
Female	162	221	152	535 (92.7)
Age (years old)				
0-2	1	11	13	25 (4.3)
3-12	0	0	6	6 (1)
12-16	3	5	6	14 (2.4)
17-25	9	28	10	47 (8.1)
26-35	32	30	25	87 (15.1)
36-45	52	65	41	158 (27.4)
46-55	25	50	30	105 (18.2)
56-65	29	34	26	89 (15.4)
>65	17	14	15	46 (8)
TOTAL	168	237	172	577 (100)

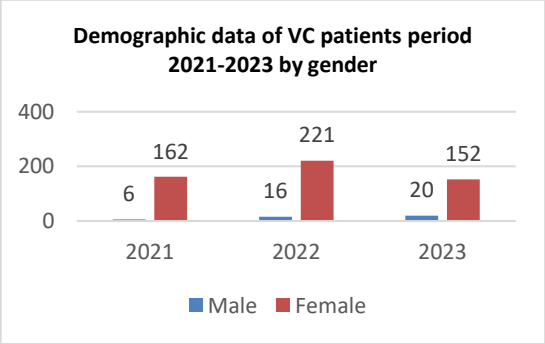

Figure 1. Demographic data of VC patients based on gender. VC patients in the past 3 years were predominantly female (92.7%) compared to male (7.3%).

The highest age range observed was 36-45 years old, which accounted for 27.4%, while the lowest was 3-12 years old, representing 1%. The demographic data of VC patients recorded in the Anatomic Pathology Department of Dr. Mohammad Hoesin RSUP between 2021 to 2023 are depicted in Figure 2.

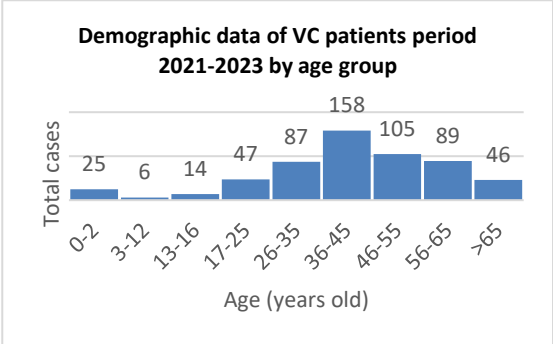

Figure 2. Demographic data of VC patients between 2021-2023 according to age group. The majority of VC patients were 36–45 years old (27.4%).

Over the past three years, VC procedure was most frequently conducted for patients with ovarian tumors, equivalent to 387 cases (66.6%). Breasts presented with tumors constituted the second most common organ subjected to this analysis (61 cases, 10.6%). Subsequently, 42 (7.3%), 24 (4.2%), 22 (3.8%), 17 (3%), 13 (2.3%), 12 (2.1%), and 2 cases (0.35%) were recorded for intestinal tumors, status margin, uterus, eyes, THT-KL, thyroid, and haematolymphoid, respectively (Figure 3).

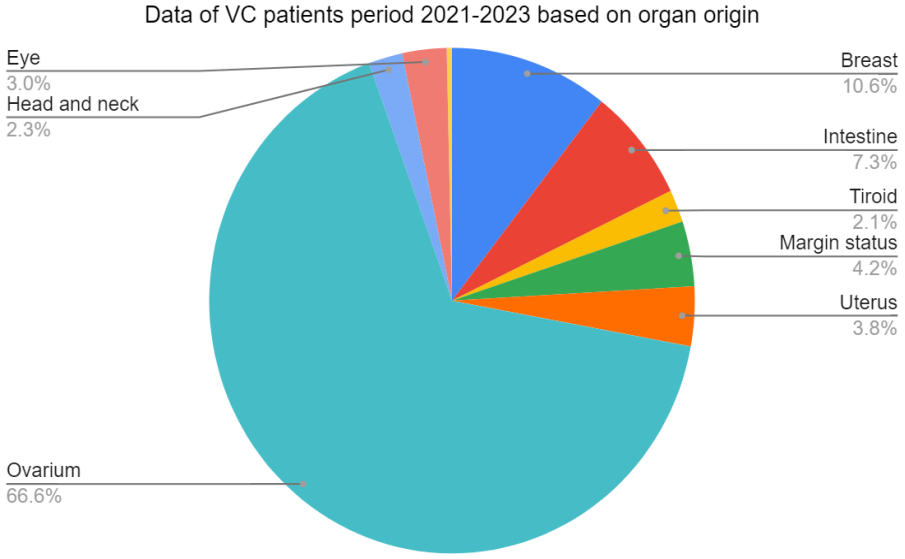

Figure 3. Distribution data of VC patients between 2021-2023 based on organ origin. Ovaries, which are part of the female reproductive system, accounted for the majority of VC procedure (66.6%).

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VC analysis is an invaluable tool for guiding surgeons during resection, ensuring that entire tumors are removed while minimizing harm to surrounding healthy tissues. Additionally, it can provide important information about the aggressiveness of tumors and the potential for metastasis, which help guide treatment decisions. Limitations commonly encountered include high dependence on the skill and experience of pathologists, along with the risk of false-negative or false-positive results. Due to these, the examination procedure is often used in conjunction with other methods to ensure accurate diagnosis and treatment planning. VC analysis is generally considered a highly accurate method for diagnosing benign, borderline, and malignant tumors (Table 2).

Table 2. Sensitivity, specificity.

	Benign	Borderline	Malignant
Sensitivity	99.7%	93.8%	98%
Specificity	99.7%	93.8%	99.6%
PPV	98.3%	93.8%	100%
NPV	98.3%	93.8%	98.3%

PPV, and NPV of VC examination.

The resulting sensitivity data, specificity, PPV, and NPV all showed values exceeding 90% based on the calculation of VC diagnostic accuracy. VC examination conducted had sensitivity, specificity, PPV, and NPV of 99.7%, 98.3%, and 98.3% for benign tumors, as well as 98%, 99.6%, 98.3%, and 98.3% for malignant tumors, respectively (Table 3).

Table 3. Comparison of VC diagnosis to the gold standard (histopathology).

VC	Histopathology		
	Malignant	Borderline	Benign
Malignant	247	0	1
Borderline	2	30	0
Benign	3	2	292

Diagnostic discrepancy

In eight cases, discrepancies were found between the definitive results of histological examination and VC diagnosis. Specifically, three, two, and other two cases diagnosed as benign, malignant, and benign tumors through VC were shown to be malignant, borderline, and other borderline tumors from histological identification. A lesion determined to be non-neoplastic based on histological analysis was initially identified as an aggressive tumor by VC.

An ovarian germ cell tumor previously identified as mature cystic teratoma from VC procedure was found to be an immature teratoma through histopathological examination, representing the most diagnostically discordant case. Mature teratoma is a benign tumor of the ovary, while immature teratoma is a malignant germ cell tumor of this same organ (Figure 4).

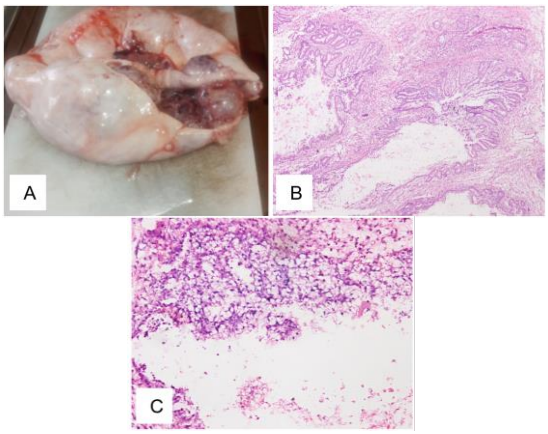

Figure 4. Histopathology of immature teratoma of the ovary. A. Neuroectodermal immature teratoma component, contains elements derived from the neuroectoderm, one of the germ layers in embryonic development. B. Rosettes composed of primitive cells commonly show a primitive neural component, which could be reminiscent of neuroepithelial rosettes. An increased nuclear-to-cytoplasmic ratio (N:C ratio) and hyperchromatic nuclei (darker nuclei). C. Presence of immature or primitive cartilage tissue in the tumor (H&E, 20 times)

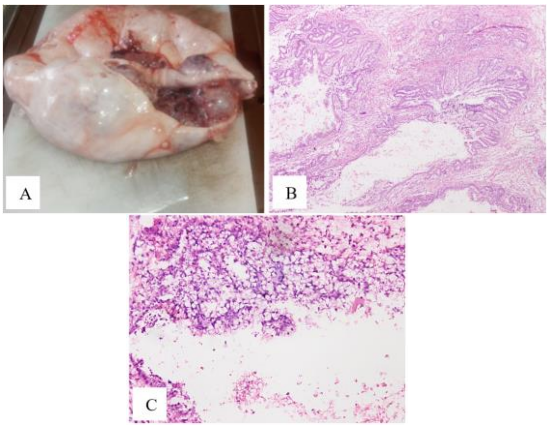

Figure 5. Ovarian mucinous carcinoma in VC fresh tissue. A. Gross tissue from the ovarian mucinous tumor. B. Extracellular mucin is a common feature of mucinous carcinomas and can be observed microscopically as areas filled with mucin between tumor cells. The tumor may show architectural complexity, with papillary projections, cribriform patterns, or micropapillae formation (H&E, 4 times). C. Signet ring cell-like appearance refers to tumor cells that contain intracytoplasmic mucin, pushing the nucleus to one side and giving the cell a characteristic shape similar to the signet ring (H&E, 20 times).

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A 25-years-old female clinically diagnosed with probable malignant mixed solid cystic ovarian tumor had a false-negative preparation for a mucinous type ovarian tumor (Figure 5). The tumor, measuring 27x22x11 cm, predominantly showed multicystic features, with some areas containing greenish-yellow fluid, while others possessed white mucous fluid and mucus.

Slides showing differences between histological and VC results were subjected to re-evaluation, where eight preparations with discordant histopathological diagnosis were independently reassessed by two pathologists. The reassessment results were further analyzed using GraphPad by Dotmatics to determine the Kappa agreement. The result produced a Weighted Kappa of 0.586, with values between 0.41 to 0.60 signifying moderate agreement.

DISCUSSION

Demographic data

The identification of high-risk disease characteristics through intraoperative VC examination can assist doctors in strategizing surgical methods. Therefore, this study evaluated 577 VC examination performed between 2021 and 2023, comparing diagnosis with the gold standard of histopathological examination.[6]

The total cases of VC examination conducted in 2021, 2022, and 2023 were 168, 237, and 172, respectively, with lower cases in 2021 being attributed to a limited number of surgical patients due to the COVID-19 pandemic. The demographic data of patients predominantly showed female equivalent to 92.7% (535/577), along with a few male at 7.3% (42/577).

Patients aged 36 to 45 years old comprised the majority of VC participants, accounting for 158 cases (27.4%), while infants aged 0 to 2 years old frequently had VC examination for Hirschsprung's disease to determine ganglion presence in the biopsy samples from 25 cases (4.3%). In investigations by Kennedy et al, focusing on VC of epithelial ovarian tumors, the mean age of patients (102/135) was 44.9 years old (SD 14.2, range 16-72 years old), with 75.6% of cases (102/135) being older than 35.[7] A VC study of Hirschsprung's disease at a tertiary hospital in Aceh, Indonesia, in 2020 showed that 55.8%, 8.8%, and 35.3% of patients were aged 0-6 years old, 7-12 months old, and over 12 months old, respectively.[8]

Tumors most frequently inspected included those affecting the ovary (66.6%), breast (10.6%), intestine (7.3%), margin status (4.2%), uterus (3.8%), eyes (2.9%), head and neck (2.3%), thyroid (2.1%), and lymph nodes (0.35%). Among 293 cases of benign tumors, 48 reports of non-neoplastic ovarian disease such as endometriosis, follicular cysts, and corpus luteum cysts were identified. This observation was in accordance with the results stated by I Gusti, which showed the examination of breast and ovarian malignant tumors as the most common VC analysis performed at the Anatomy Department of FK UNUD/RSUP Sanglah, Denpasar.[9] Conversely, the study conducted by Anes Mohamed et al on 3675 VC examination identified that 854, 643, 573, and 516 cases were related to head and neck, lymph nodes, gynecology, as well as the thoracic region, respectively.[10,11]

Diagnostic Discrepancy

Eight of the 577 cases of VC analysis conducted showed diagnostic discordance with the definitive results of histopathological examination (Table 2). These discordant cases entirely originated from the ovary, comprising two, five, and one cases of teratoma germ cell tumor, mucinous ovarian tumor, and endometriosis, respectively. Moreover, VC analysis accuracy generally exceeded 99%, and diagnostic agreement or concordance identified in cases of potential malignancy has been compared with previous studies. Primary ovarian mucinous borderline tumor/carcinoma and low-grade serous carcinoma represented the majority of discordant cases.[12] Discordance rates between VC and histopathology, based on the investigation performed by Chinelo in 2022, showed minimal differences (2.0% [33/1,607] vs 2.7% [40/1,472], P=0.24).[13]

Benign, borderline, and malignant tumors subjected to VC analysis showed sensitivity, specificity, high PPV, and NPPV values of 99.7%, 93.8%, and 98%, 99.6%, 93.8%, and 9.97%, 98.3%, 93.8%, and 100%, as well as 98.3%, 93.8%, and 98.3%, respectively. Another study reported variances in the sensitivity and specificity values of malignant (96% and 100%) and borderline tumors (83% and 99%).[14]

VC examination performed for a female patient identified an ovarian tumor that seemed to be borderline, while subsequent histopathological diagnosis showed a higher degree of severity/carcinoma. This was consistent with the study by Ria et al, which reported 90% accuracy for VC examination

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conducted on ovarian tumors. The sensitivity values for borderline, benign, and malignant tumors were 78%, 90%, and 91%,[15] suggesting intraoperative VC as a valuable tool for predicting malignancy.

Patients with ovarian borderline tumors need to be aware of mucinous tumor aggression, particularly in older age.[16] Pathologists face challenges in identifying ovarian tumors, specifically the mucinous types, and great caution is required in the process. Jeong Yeol Park et al investigated variables associated with improving the conclusive identification of mucinous ovarian tumors and VC diagnostic accuracy. The conducted study identified tumor size >12 cm, multilocular tumor, mixed tumor histology results, and the presence of solid components in the tumor as independent risk factors for improving diagnostic accuracy.[17] Mohamed et al found that diagnostic concordance was influenced by sampling errors (27%), sample limitations (45%), and interpretation errors (27%).[11]

Histopathological features of the tumor type known as immature teratoma comprise elements derived from multiple germ cell layers, such as ectodermal, mesodermal, and endodermal tissues. The specific features include the presence of immature or incompletely differentiated tissues, such as primitive neuroectodermal elements, cartilage, or glandular structures. Moreover, surgery commonly performed in the form of tumor resection, serves as the primary treatment for teratoma, and complete surgical removal of the tumor is essential to achieve optimal results and minimize the risk of recurrence. Teratoma, particularly immature types, are generally resistant to chemotherapy and radiation therapy, because these modalities may be less effective due to teratoma heterogeneity and complexity, as well as the presence of immature components lacking adequate response to standard treatments.[18,19] The size and diverse macroscopic appearance of immature teratoma tend to cause diagnostic discordance, posing challenges for pathologists, while necrosis, butter, as well as tissues including blood, butter, bone, hair, and nails contribute to the complexity encountered.[9,20]

In this study, patients with discordant results between VC and histopathological examination were subjected to re-evaluation. Two pathologists re-assessed eight discordant VC slide preparations by conducting analysis using Dotmatics' GraphPad to determine Kappa agreement, where the obtained value was 0.586, signifying moderate agreement.[21] This result was consistent with the investigations conducted by Kediya et al, which showed a higher Kappa statistical value for histopathological examination compared to the diagnostic accuracy value obtained from intraoperative VC analysis of gynecological neoplasms.[22] Accurate diagnosis is facilitated by the use of proper grossing methods that minimize sampling errors, while the differential algorithm integrated with clinical data should be continuously improved to prevent errors.[14]

Studies by Sharon B. Sams and Joshua also identified differences in the final results of 24 (2.3%) and 48 (4.6%) VC examination cases, exploring various factors including procedural errors and less representative samples.[11,23] Achieving an accurate diagnosis requires good clinical expertise, beginning with proper grossing sample selection and VC tissue analysis, followed by precise diagnosis from pathologists.

In conclusion, this study identified VC examination as a valuable tool with high sensitivity and specificity in supporting intraoperative diagnosis. VC examination was found to promote rapid determination of the correlation between features of tissue samples obtained during surgery with benign or malignant criteria. Therefore, a comprehensive evaluation of various factors capable of contributing to the accuracy of this diagnostic procedure was recommended.

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Conflict of interests
The authors reported no potential conflicts of interest.

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