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Suitability of the Cellient™ cell block method for diagnosing soft tissue and bone tumors

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BACKGROUND: The diagnosis of tumors of soft tissue and bone (STB) heavily relies on histological biopsies, whereas cytology is not widely used. Cellient™ cell blocks often contain small tissue fragments. In addition to Hematoxylin and Eosin (H&E) interpretation of histological features, immunohistochemistry (IHC) can be applied after optimization of protocols. The objective of this retrospective study was to see whether this cytological technique allowed us to make a precise diagnosis of STB tumors.

METHODS: Our study cohort consisted of 20 consecutive STB tumors, 9 fine-needle aspiration (FNAC) samples, and 11 endoscopic ultrasonography (EUS) FNACs and included 8 primary tumors and 12 recurrences or metastases of known STB tumors.

RESULTS: In all 20 cases, H&E stained sections revealed that diagnostically relevant histological and cytological features could be examined properly. In the group of 8 primary tumors, IHC performed on Cellient™ material provided clinically important information in all cases. For instance, gastrointestinal stromal tumor (GIST) was positive for CD117 and DOG-1 and a PEComa showed positive IHC for actin, desmin, and HMB-45. In the group of 12 secondary tumors, SATB2 was visualized in metastatic osteosarcoma, whereas expression of S-100 was present in 2 secondary chondrosarcomas. Metastatic chordoma could be confirmed by brachyury expression. Two metastatic alveolar rhabdomyosarcomas were myf4 positive, a metastasis of a gynecologic leiomyosarcoma was positive for actin and estrogen receptor (ER) and a recurrent dermatofibrosarcoma protuberans expressed CD34.

CONCLUSION: In the proper clinical context, including clinical presentation with imaging studies, the Cellient™ cell block technique has great potential for the diagnosis of STB tumors.

KEYWORDS
cell block, Cellient, cytopathology, immunochemistry, soft tissue and bone tumors

1 INTRODUCTION

Soft tissue and bone (STB) tumors are rare malignancies, which comprise approximately 2% of all neoplasms. Because of this low incidence, patients with STB tumors are usually referred to expert sarcoma centers, where multidisciplinary teams, according to well-established protocols and latest developments, can perform diagnostic procedures and treatment. The clinical diagnosis of primary STB tumors relies on imaging studies and an adequate biopsy. Imaging, in particular with computed tomography (CT) and magnetic resonance imaging (MRI), provides insight into the location, size, margins, and tissue composition and heterogeneity of STB tumors. Although cytology has been applied in just a few sarcoma centers, the primary diagnosis of STB tumors is usually made on histological (needle or open) biopsies, since these malignancies are morphologically heterogeneous and several histological types have overlapping microscopic features. Moreover, for a conclusive diagnosis of STB tumors, additional immunohistochemistry (IHC) and molecular pathology (fluorescent in situ hybridization (FISH),...
polymerase chain reaction (PCR), and next generation sequencing (NGS) often have to be administered, requiring special expertise.

Typing and grading of STB tumors is mandatory for treatment decisions. In this clinical context, in our and most other sarcoma teams, cytology is only applied in selected cases. Firstly, in cases with an established diagnosis of the primary STB tumor, cytology can be effectively used to diagnose recurrent or metastatic sarcoma. Secondly, for deep-located STB tumors, cell material can be collected by fine-needle aspiration (FNAC) during endoscopic ultrasonography (EUS-FNA) or by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS). In our sarcoma team there is ample experience with these techniques.\textsuperscript{2,4,8} Compared with histological biopsies, it is more easy to sample different tumor areas with fine-needle aspiration (FNA), and this may result in increased diagnostic accuracy, in particular when dealing with STBs with heterogeneous features on clinical imaging (CT and MRI).

Several different cell block methods can be used to process cell material thus collected.\textsuperscript{9} As an adjunct to routinely prepared smears or cell sediments, cytoblock techniques allow the application of IHC and molecular methods, expanding the diagnostic armamentarium. For this purpose, we and others\textsuperscript{8,10–14} have used the Cellient\textsuperscript{TM} automated cell block system, by which cytotechnicians can make an automated cell block within 1 hour, albeit with higher costs than that of traditional cell block techniques. As described previously, with the Cellient\textsuperscript{TM} method, using methanol fixation instead of formalin, a broad array of diagnostically important antibodies can be applied to IHC after optimization of IHC protocols. In clinical cytology, the Cellient\textsuperscript{TM} method has been used successfully for the characterization of tumor cells in serous fluids and FNAC material, for example, to characterize different carcinoma types or to diagnose metastatic melanoma.\textsuperscript{12}

In this article, we report our first experience on the suitability of the Cellient\textsuperscript{TM} method to diagnose several types of STB tumors, 8 primary lesions (5 of which were gastrointestinal stromal tumors) and 12 secondary recurrences or metastases, applying 9 diagnostically relevant antibodies that were not described in our earlier article of the Cellient\textsuperscript{TM} method.

### 2 Material and Methods

#### 2.1 Ethics Statement

The study met the criteria of the code of conduct for responsible use of human tissue that is used in the Netherlands (Dutch federation of biomedical scientific societies; http://www.federa.org).
2.2 | Cell samples

Cell samples of aspirations from soft tissue and bone tumors processed with the Cellient™ processor (Hologic, Marlborough, Massachusetts) between 2013 and 2016 were retrieved from the archives of the cytology laboratory of the pathology department of University Medical Center Groningen. Our cohort consisted of 20 consecutive cases, shown in Table 1, and included 12 EUS guided aspirations of deep-seated tumors (in the abdomen, retroperitoneal space, and mediastinum) and 9 FNAC specimens of superficial lesions. All but 1 EUS guided aspirations were performed with an EProCore needle (ECHO-HD-22-C; Echo Tip Ultra; Cook Medical, Bloomington, Indiana). In all EUS procedures and most FNAC aspirations, specimen cellularity had been checked on site by our cytotechnicians and in case of low cellularity, repeated aspirations were done. The study cohort comprised 16 soft tissue tumor cases (5 were gastrointestinal stromal tumors) and 4 bone tumor cases (2 chondrosarcomas and 2 osteosarcomas). For evaluation of diagnostic performance, the cohort was divided in 2 groups, 8 primary lesions, and 12 secondary lesions (metastases or recurrences of tumors of which the histologic diagnosis was known). Histologic follow-up was available for all primary tumors, allowing correlation of cytological and histological diagnosis.

2.3 | Cellient™ cell block technique

Before being loaded into the Cellient™ processor (Figure. 1A,B), materials were washed in 1 mL Cytolyt™ Wash, centrifuged at 1000 g for 5 minutes, dissolved in 20 mL PreservCyt™ fluid and fixed for 20 minutes. One drop of the cell sediment was used to

### Table 2

| Antibody | Clone type | Clone | Manufacturer | Dilution | Pretreatment |
|----------|------------|-------|--------------|----------|--------------|
| Actin-SMA | Monoclonal | 1A4 | Ventana | R.T.U | No |
| Beta-catenin | Monoclonal | 14 | Ventana | R.T.U | CC1 52 min |
| Brachyury | Monoclonal | EPR18113 | Abcam | 1:400 | CC1 36 min |
| Caldesmon | Monoclonal | h-CD | Dako | 1:800 | No |
| CD-117 | Polyclonal | C-KIT | Dako | 1:100 | No |
| CD-34 | Monoclonal | QBEND10 | Ventana | R.T.U | CC1 92 min |
| CK-AE1/3 | Monoclonal | AE1/AE3 | Ventana | R.T.U | CC1 36 min + protease 4 min |
| Desmin | Monoclonal | DE-R-11 | Ventana | R.T.U | CC1 64 min |
| DOG1 | Monoclonal | SP 31 | Ventana | R.T.U | |
| ER | Monoclonal | SP-1 | Ventana | R.T.U | No |
| HMB-45 | Monoclonal | HMB45 | Ventana | R.T.U | No |
| myf-4 | Monoclonal | LO26 | Monosan | 1:25 | CC1 64 min |
| S-100 | Monoclonal | 4C4.9 | Ventana | R.T.U | No |
| SATB2 | Monoclonal | 4B10 | Abcam | 1:100 | CC1 64 min |

CC1, cell conditioning solution (Ventana), pre-treatment buffer, pH 8.4.
prepare a Giemsa-stained smear. In addition, 6 drops of the cell sediment were washed for 20 minutes in 1 mL Cytolyt Wash™ (Holotigic), a low-dose methanol-based solution used to lyse erythrocytes and dissolve mucus. From this sediment, a Papanicolaou-stained microscopic thin layer slide was prepared with the ThinPrep T5000 processor. The remaining part of the cell suspension was rinsed twice in Cytolyt Wash™ solution and centrifuged again for 5 minutes at 1200 g, after which the pellet was fixed with PreservCyt™ fluid for 20 minutes before the sample vial with PreservCyt™ was put in the automated Cellient™ processor. The Cellient™ Automated Cell Block System is fully automated. It creates a paraffin-embedded cell block in 1 hour by means of a controlled vacuum that concentrates a layer of cells on a specially designed filter. Dehydrating and clearing reagents, including propranolol and xylene, are vacuum-drawn through the sample, which is subsequently embedded in paraffin and finished in an additional layer of paraffin; this makes it ready for histological sectioning. The vacuum-assisted filtration concentrates available cells within the final paraffin block. Eosin staining is used for visualization of the cell layer during sectioning. During sectioning of the Cellient™ cell blocks 10 paraffin sections of 4-mm thickness were prepared, and these were mounted on aminopropyltriethoxysilane (APES)-coated microscopic slides. One section was routinely stained with Hematoxylin and Eosin (H&E) for microscopic evaluation of specimen cellularity. The remaining unstained slides were available for IHC.

2.4 | Immunohistochemistry

The 14 antibodies (13 monoclonal, 1 polyclonal) applied in this study, including their commercial source, clone, and working dilution, as summarized in Table 2. Five diagnostically relevant antibodies had been evaluated in our earlier study of the Cellient™ method (CD117, AE1/3, ER, HMB-45, S-100). The 9 additional antibodies used to diagnose the STB tumors in this cohort were actin, beta-catenin, brachyury, caldesmon, CD-34, desmin, DOG-1, myf4, and SATB2. All IHC stains were performed in the Benchmark Ultra automated immunostainer (Ventana, Tuscon, Arizona) using the Ultraview detection system and validated by testing proper dilution of the antibody, need for CC1 antigen retrieval, and need for an 8 minutes amplification step in the IHC staining protocol, respectively (Table 2). All antibodies had been tested with at least 3 different Cellient™ cell blocks prepared from 3 different specimens. IHC results obtained with Cellient™ cell blocks were compared with IHC results obtained with corresponding formalin-fixed, paraffin-embedded (FFPE) tissue tumor material from the same patient as reference standard. Several antibodies required antigen retrieval with CC1 (cell conditioning buffer, pH 8.4) for optimal staining. CC1 with protease pretreatment proved to give the best results for cytokeratin antibody AE1–3. For all IHC staining, the Ventana Ultraview DAB detection kit was used with an amplification step of 8 minutes. Hematoxylin was used as a counterstain.
3 | RESULTS

In all 20 cases, the H&E stained sections of the Cellient™ material contained small tissue fragments. H&E histology of these small tissue fragments (microbiopsies) revealed that diagnostically relevant histological and cytological features could be examined properly, as shown in Figure 2. Fragments of desmoid fibromatosis consisted of collagen rich tissue with haphazardly arranged fibroblastic cells with round nuclei, nucleoli, and tapering eosinophilic cytoplasm (Figure 2A). Fragments of a grade 2 myxoid chondrosarcoma contained tumor cells with moderately atypical, hyperchromatic, single and double nuclei embedded in myxochondroid matrix (Figure 2B), whereas cellular fragments with pleomorphic and hyperchromatic tumor cells were encountered in recurrences of pleomorphic undifferentiated sarcoma and radiation sarcoma (malignant peripheral nerve sheath tumor (MPNST)) (Figure 2C,D).

In the group of 8 primary tumors, we specifically diagnosed 5 spindle cell gastrointestinal stromal tumors (GISTs) by positive IHC for both CD117 and DOG-1 (B) in GIST. Desmin (C) and HMB-45 (D) in PEComa. SMA (E) and caldesmon (F) in leiomyosarcoma. Nuclear staining of osteosarcoma cells with SATB2 (G), S-100 (H) in chondrosarcoma, brachyury (I) in chordoma, myf4 (J) in alveolar rhabdomyosarcoma, and ER (K) in gynecologic leiomyosarcoma. CD34 (L) in dermatofibrosarcoma protuberans (original ×200) [Color figure can be viewed at wileyonlinelibrary.com]
The Cellient™ is a fully automated device that produces a cell block from aspirates and excisions/resections from the same patient. In both groups (primary and secondary tumors), IHC results in Cellient™ slides were concordant with those obtained in FFPE tumor biopsies or excisions/resections from the same patient.

Figure 3 depicts IHC results of all antibodies applied: CD117 (Figure 3A) and DOG-1 (Figure 3B) in GIST, desmin (Figure 3C) and HMB-45 (Figure 3D) in PEComa, SMA (Figure 3E) and caldesmon (Figure 3F) in leiomyosarcoma, SATB2 in osteosarcoma (Figure 3G), S-100 in chondrosarcoma (Figure 3H), brachyury in chordoma (Figure 3I), myf4 in alveolar rhabdomyosarcoma (Figure 3J), ER (Figure 3K) in metastatic gynecologic sarcomas, and DOG-1 (Figure 3L) in GIST, desmin (Figure 3C) and HMB-45 (Figure 3D) in PEComa, SMA (Figure 3E) and caldesmon (Figure 3F) in leiomyosarcoma, SATB2 in osteosarcoma (Figure 3G), S-100 in chondrosarcoma (Figure 3H), brachyury in chordoma (Figure 3I), myf4 in alveolar rhabdomyosarcoma (Figure 3J), ER (Figure 3K) in metastatic gynecologic sarcomas, and CD34 (Figure 3L) in dermatofibrosarcoma protuberans.

4 | DISCUSSION

The Cellient™ is a fully automated device that produces a cell block within 1 hour based on a standardized protocol. This allows rapid diagnosis on the same day the specimen arrives in the lab instead of the following day, which is convenient in selected cases. A methanol-based PreservCyt™ solution is used instead of formalin. Several research groups have mentioned that the cellularity of Cellient™ material is at least comparable to that in traditional cell blocks, whereas cytomorphic details, in particular chromatin structure, appear to be better. Advantages and disadvantages of commonly used cell block methods including Cellient™ have been amply reviewed by Jain et al.9 and are summarized in Table 3. We have noted that the Cellient™ cell blocks often contain small tissue fragments. In addition to H&E interpretation of histological features, IHC and molecular methods, for example, FISH or NGS, can be applied.8 In the cytology laboratory, immunostaining can be applied to cell smears, ThinPrep specimens, cytospin specimens, and cell blocks. In a UK NEQUAS quality control study, testing commonly used antibodies for a diagnosis of carcinoma, mesothelioma, melanoma, and lymphoma, it was found that the highest sensitivity was provided by cell blocks, followed by cytospin specimens, liquid-based cytology slides, and cell smears.13

Although cellular DNA and RNA are well preserved by methanol fixation, at the protein level, IHC protocols that are routinely used for FFPE material, have to be optimized and validated. We8 and Sauter et al.13 have extensively tested many different antibodies for Cellient™ material using the automated Ventana Benchmark immunostainer. In our initial study published in 2013, we showed that IHC performed on Cellient™ cell blocks could be applied to diagnostic algorithms that proved to be helpful in the discrimination of major tumor types (carcinoma, lymphoma, melanoma, and germ cell tumors), discrimination of carcinoma subtypes (adenocarcinoma, squamous-cell carcinoma, and neuroendocrine carcinoma), and determination of primary tumor site (eg, lung and breast) in cases of metastatic carcinoma. Notably, in a consecutive series of 100 cases, additional and clinically relevant information was obtained in 25% of serous fluid specimens and 29% of FNA specimens.8

### TABLE 3  Comparison of commonly used cell block methods, as reviewed by Jain et al.9

| Method   | Advantage                                                                 | Disadvantage                                                                 | Utility                                                                 | IHC                                                                 | Molecular studies                                      |
|----------|---------------------------------------------------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------|----------------------------------------------------------------------|--------------------------------------------------------|
| Agar method | Inexpensive Better orientation of cell block                              | Inconvenient heat treatment process Heat-related artefacts possible, if not cooled as recommended | For any fluid or FNA                                                   | Optimum results for cytoplasmic and nuclear antigens               | Suitable                                               |
| Histogel method | Good cellular preservation and architecture                              | Tedious process as HistoGel needs to be converted and maintained in liquid state Possible heat-related artefacts | Useful in specimens with no visible sediment after centrifugation     | Suitable                                                           | Suitable                                               |
| Collodion bag method  | Good cellular yield                                                      | Time-consuming preparation of bags Toxic ether fumes for storage             | Friable tissues and fragments, specimens of scanty cellularity         | Appropriate results                                                 | Suitable                                               |
| Cellient method  | Uniformly distributed cells Improved cellular architecture and nuclear features Consistent results Automated method with reduced procedural time No cross contamination Minimal cell loss | Expensive machines and consumables Requires trained staff for cutting thin sections | Limited studies Useful in low-cellularity specimens Useful in cervical LBC | Good results with optimized IHC protocols, adjusted to methanol fixation (see Refs. 8 and 13) | High quality of DNA and RNA                           |

Abbreviations: FNA, fine needle aspiration; IHC, immunohistochemistry; LBC, liquid-based cytology.
To our knowledge, this is the first report on the use of Cellient® cell blocks for the diagnosis of soft tissue and bone tumors. We stress that, in our and most sarcoma centers, cytology is only rarely applied for diagnosing tumors of soft tissue and bone. For the tumors in this series, EUS-FNA was tried to render a diagnosis of deep-seated primary tumors and FNA was used for superficially located metastatic or recurrent tumors of which the histologic diagnosis was known. We showed that by combining clinical presentation (including imaging studies), H&E morphology, and IHC, a diagnosis could be made in all 20 consecutive cases of tumors of soft tissue and bone. We evaluated 14 antibodies, 9 of which were not tested in our initial study. After optimization of factors influencing IHC results (in particular antigen retrieval conditions, amplification steps in the detection system kit) we managed to obtain excellent staining results for both cytoplasmic (eg, the smooth muscle markers, actin, desmin, and caldesmon) as well as nuclear antigens (eg, brachyury, myf4, and SATB2). In all 16 cases (all 8 primary tumors and 8 secondary tumors) where IHC was applied, a specific diagnosis could be made. For instance, brachyury, myf4, and SATB2, which are markers for notochordal, myogenic, and osteoblastic cell differentiation, respectively, allowed or confirmed a diagnosis of chordoma, alveolar rhabdomyosarcoma, and osteosarcoma.

Finally, cost considerations and budgetary constraints will determine the extent to which cytology laboratories use the rapid automated processing or more time-consuming traditional manual FFPE method to prepare cell blocks for H&E, IHC and/or FISH. Costs of the Cellient® technique include purchase (50 000 US$) and reagents (10 US$ per specimen). Although the cost of the Cellient® block technique is higher than that of a traditional cell block technique, we estimated that saved technician time is 30 minutes per specimen, using the time required to prepare an agar cell block as a reference standard. However, in our opinion, the cost of a new laboratory technique should be judged in the context of total cost of patient health care, including reduction of other diagnostic tests and patient life years saved, a cost analysis which is beyond the scope of this article.

In summary, we have shown that routine H&E staining and IHC of cell material processed with Cellient® processor has the potential to accurately diagnose tumors of soft tissue and bone. In all 20 consecutive cases, important clinical information was provided, which translated into improved patient care. However, due to the small sample size, statistical analysis was not feasible, and a future study, testing appropriate antibodies on a larger number of cases, is needed to assess the real value of this method.

CONFLICT OF INTEREST
The authors have no conflict of interest.

DISCLOSURES
The authors made no disclosures.

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