The Efficacy of Using the Tissue Fragments Present in Cervical Scrapes for the Histologic Diagnosis of Cervical Neoplasia

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Abstract: Cervical scrapes to diagnose cervical neoplasia, collected by the clinician with brushes, are sent to the Leiden Cytology and Pathology Laboratory (LCPL) in vials containing BoonFix, a noncross-linking coagulant fixative. Because the residual material left in the vials contains tissue fragments with important diagnostic information, we stored the residual material in our archives. The tissue fragments can be mummified and archived in commercially available histology cassettes. We can produce paraffin sections thereof. Immunostaining is beautiful on serial paraffin sections cut from these blocks.

We experienced that it is important to leave the brush in the vial such that all tissue fragments can be used for histologic diagnosis. In order to optimize the system, tissue fragments left in the endocervical part of the brush are removed in a paint shaker.

We illustrate this principle of recovering mummified tissue fragments in a false negative case with cytology containing many undiagnosable collapsed tissue fragments. This case shows clearly the efficacy of using the tissue fragments present in cervical scrapes for the histologic diagnosis of cervical neoplasia.

Keywords: Cervical scrapes, BoonFix, cervical cancer.

INTRODUCTION

Cervical scrapes to diagnose cervical neoplasia, collected by the clinician with brushes, are sent to the Leiden Cytology and Pathology Laboratory (LCPL) in vials containing BoonFix, a noncross-linking coagulant fixative. Because the residual material left in the vials contains tissue fragments with important diagnostic information [1-4], we store the residual material. The vials take abundant space in our wet archive. Therefore we developed a dry storage method using commercially available plastic histocassettes, which are easy to label and can be stored in our dry archive.

The cassettes containing the mummified specimen are archived in 46.5 × 25.5 × 5.5 cm boxes that can each contain 400 cassettes. The Leiden laboratory can archive 4,200 cassettes in the same space used for 400 vials. Further examination of the putative important tissue fragments is possible by placing the mummified specimen in BoonFix®, through which the original morphology and staining is restored. We experienced that ThinPrep slides made from the material still present in the archival vials have the same quality as the original ones with clear chromatin details. We can also make paraffin sections, from tissue blocks prepared with an agar method. Immunostaining is beautiful on serial paraffin sections cut there off.

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In this paper we present the importance of processing mummified tissue fragments in a false negative case, with the aim to illustrate the efficacy of using the tissue fragments present in cervical scrapes for the histologic diagnosis of cervical neoplasia.

MATERIAL AND METHODS

After selecting the histocassette, the mummified material was placed in a vial containing BoonFix. It was left in the vial for 24 hours. A drop of Eosin is added to the BoonFix suspension to visualize the tissue fragments. Next the suspension is filtered. The red specimen on the filter is clearly visible. The wet filter is folded, placed in a histocassette and processed to paraffin blocks.

RESULTS

In the paraffin section prepared from the mummified archival material many large viable cancerous tissue fragments were observed with dark blue staining nuclei (Figure **1**). In addition, large red staining necrotic tissue fragments were present. At a higher magnification (Figure **2**), the architecture of the cancerous tissue was precisely visualized. It becomes evident that we are dealing with a case of squamous cell carcinoma.

The efficacy of leaving the collecting brush in the vial and placing it in a paint shaker becomes clear when one compares the brush before and after the paint shaker procedure as is illustrated in Figure **3**: Left

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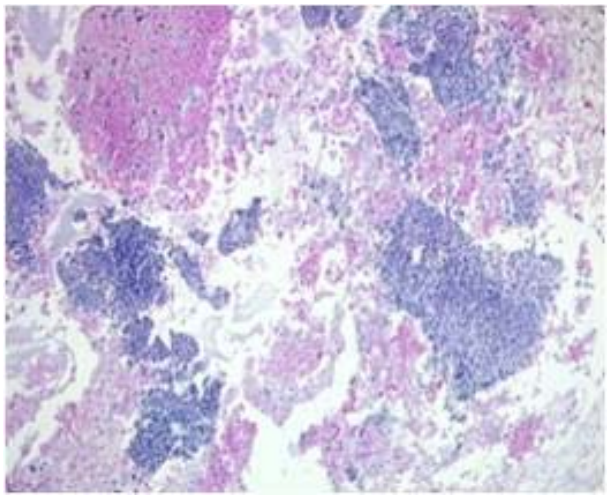

Figure 1: In the paraffin section prepared from the mummified archival material many large viable cancerous tissue fragments were observed with dark blue staining nuclei. In addition, large red staining necrotic tissue fragments were present.

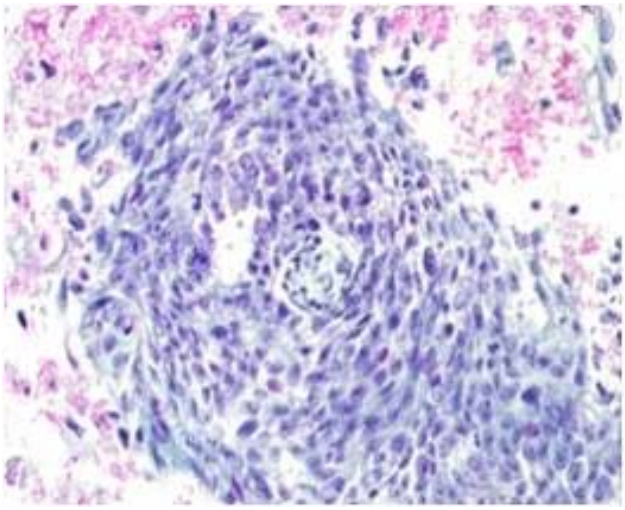

Figure 2: At a higher magnification, the architecture of the cancerous tissue was precisely visualized. It becomes evident that we are dealing with a case of squamous cell carcinoma.

before and right after the paint shaker procedure. Particularly from the endocervical part of the brush, cancerous material is retrieved.

In this paper, a false negative case of squamous cell carcinoma is presented. The original ThinPrep slide contained a large number of undiagnosable tissue fragments (Figure **4**). In addition, blood and necrotic material were prominent. The cytologic diagnosis was cancer negative. We became aware that this was a false negative case because cancer was diagnosed by the gynecologist one year later. The mummified material of the original sample was present in our archive and could be used to prepare paraffin sections.

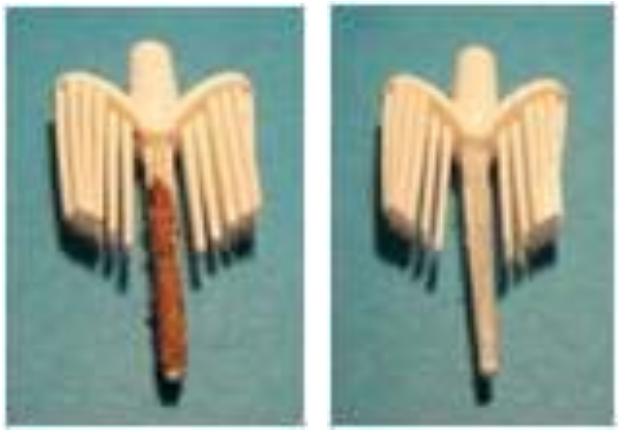

Figure 3: The efficacy of leaving the collecting brush in the vial and placing it in a paint shaker is illustrated. Left before and right after the paint shaker procedure. Particularly from the endocervical part of the brush, cancerous material is retrieved.

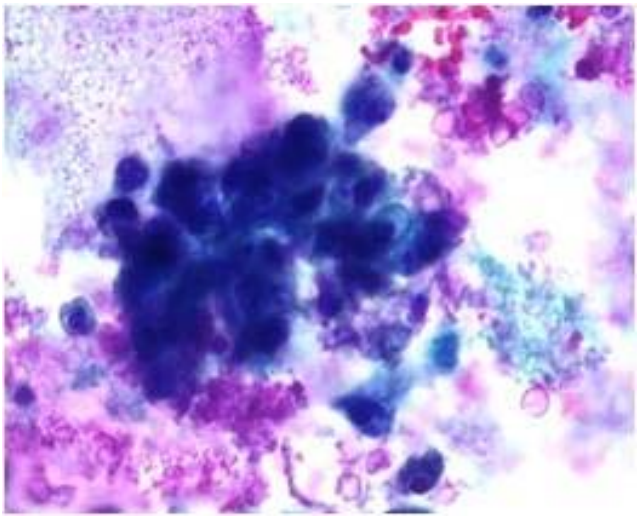

Figure 4: Undiagnosable collapsed tissue fragments blood and necrotic material in the original ThinPrep slide erroneously signed out as negative.

DISCUSSION

The FDA guidelines for ThinPrep sampling dictate to send the vial to the laboratory without the collecting brush which is ultimately thrown in the wastebasket by the sampling clinician. However, we found that it would be more diagnostic-efficient if the vial was received with the brush in it, allowing removal of the tissue fragments from the brush and embed these in paraffin. From these histology blocks, serial sections can be cut for routine and immunostaining.

From the 66,000 ThinPrep samples sent to the Leiden laboratory for a cytology diagnosis in 2007, 233 were selected for brush histology based on the presence of collapsed tissue fragments in the ThinPrep

cytology slide. Of all 233 brush histology cases the follow-up diagnoses were retrieved. For comparison, of 1,386 routinely diagnosed cases in 2007, follow-up data were also collected. The pathology reports of the colposcopic biopsies of the patients were collected from the Dutch National Pathology Database, the PALGA. All histology diagnoses were stratified into CIN 0 for cases without (pre)neoplasia, CIN 1, CIN 2, CIN 3, and carcinoma. Of the 233 brush histology cases 9% proved to harbor invasive carcinoma versus 2.1% in the routine group of 1,386 cases. For CIN 3 these scores were 48.5% versus 19.6%.

In closely analyzed cases in which the brush histology was not confirmed, the colposcopic biopsies proved to be insufficient. In one case with a brush histology diagnosis of CIN 3, the colposcopic biopsy was CIN 0 but invasive carcinoma was established in 2008. One case of cervical endometriosis was erroneously diagnosed in the brush cytology as adenocarcinoma.

In the Leiden laboratory, the vial with the brush is placed in a paint shaker such that all diagnostic material is removed from the brush. In 2009, 45,326 cervical specimen contained a brush (Brush+) and 227 did not contain a brush (Brush–). All 45,553 samples were part of the Dutch national screening program. The unsatisfactory slides were microscopically subclassified into too bloody, too few cells, and too thick. The brush status of the vial was entered into a quality control database but was not available for the screening cytotechnologist. In the Brush+ and Brush− group respectively 1.4‰ and 35.2‰ had an unsatisfactory cytology. In the 63 Brush+ unsatisfactory cases a diagnosis was not rendered. Of these 63 Brush+ unsatisfactory slides 11.11% was too bloody, 77.8% contained too few cells, and 11.11% was too thick. All 8 unsatisfactory Brush− slides contained too few cells to warrant a diagnosis. If in *all* 45,553 ThinPrep® samples the brush would have been removed 1,603 women (instead of 71) would have had an unsatisfactory cytology. These data refute the wisdom of the FDA guidelines. It is clear that for 63 women it was worth that we were disobedient and that the brushes were kept into the ThinPrep® collection vial such that a diagnosis could be given on their samples.

In the case of squamous cell carcinoma presented in this paper, we show that the squamous cell carcinoma fragments can still be retrieved from the original mummified tissues. The microphotographs (Figures **1** and **2**) show that it is not difficult to render the correct diagnosis on the tissue sections, and the photographs taken of the collecting brush clearly visualize how important it is to use a paint shaker to remove the cancerous tissue from the endocervical part of the sampling brush.

CONCLUSION

In this paper we have shown the efficacy of using tissue fragments in cervical scrapes for the histologic diagnosis of cervical neoplasia.

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