VACUUM-BASED PRESERVATION OF COLORECTAL CANCER SPECIMENS: A COMPARISON WITH FORMALIN FIXATION

Jean-François Fléjou, Samah El-Naderi, Pascale Cervera.

Department of Pathology, Saint-Antoine Hospital, AP-HP,

Medical School Pierre et Marie Curie, Paris, France

jean-francois.flejou@sat.aphp.fr

INTRODUCTION

In Pathology, an alternative to immediate fixation in neutral buffered formalin (NBF) is vacuum sealing and cooling (VSC) ("TissueSAFE" system) *(Bussolati G et al. Virchows Archiv 2008;452:229-231)*. There have been few evaluation of VCS.

Tissues that have been processed with VCS are not only used for standard morphological techniques, but also for molecular tests, mandatory in various types of solid tumours, including colorectal cancer.

MATERIAL AND METHODS

We assessed microsatellite instability (MSI), KRAS mutations and BRAF mutations in colorectal cancers, conserved with VCS before fixation (51 cases with surgery in a hospital distant from our centre), or immediately fixed in NBF (56 cases with surgery in a hospital distant from our centre).

DNA was extracted from paraffin embedded tissue with a standard procedure in both groups.

MSI was assessed by MLH1-MSH2 immunohistochemistry and PCR (pentaplex mononucleotidic assay); KRAS and BRAF were screened by multiwell-plate based real-time PCR (LightCycler 480, Roche), confirmed by Sanger sequencing.

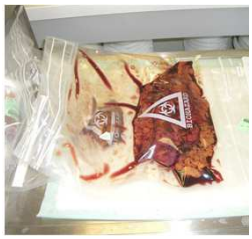


Figure 1 : The TissueSAFE system

RESULTS

There was no difference in both groups regarding morphological analysis (Figure 2 shows H&E staining of a colorectal cancer specimen after VCS).

DNA extraction was possible in all cases.

Immunohistochemistry was interpretable in all cases, with 4 negative cases in the VCS group (4 MLH1), 11 negative cases in the NBF group (10 MLH1 and 1 MSH2), and a 100% correlation between immunohistochemistry and PCR (Figure 3 shows a VCS case with preserved expression of both proteins).

KRAS and BRAF mutations were detected in 21 and 1 cases in the VCS group and 16 and 7 cases in the NBF group, respectively (Figure 4).

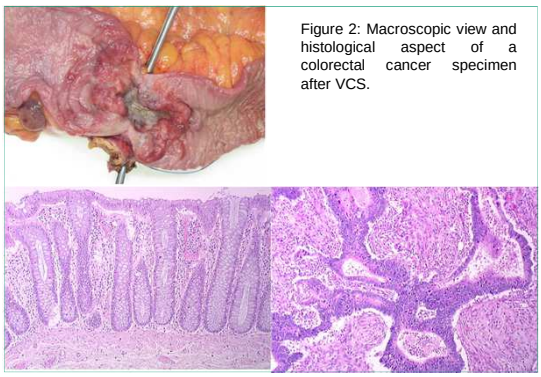


Figure 2: Macroscopic view and histological aspect of a colorectal cancer specimen after VCS.

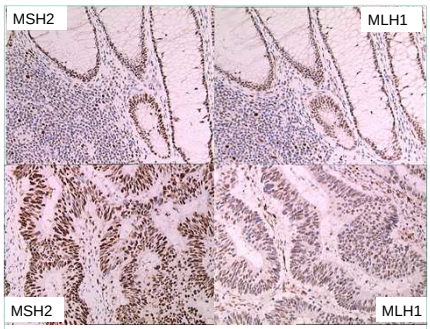
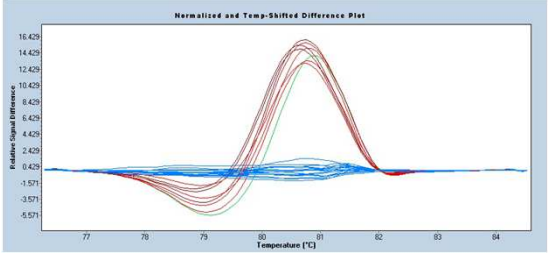
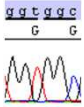


Figure 3: colorectal cancer specimen after VCS, showing positive MLH1 and MSH2 immunostaining both in the normal mucosa (upper) and in the cancer (lower).

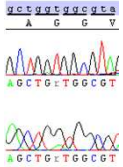
Figure 4: Normalized and Temp-shifted difference plots showing KRAS wild type and mutated colorectal cancers fixed after VCS (Lightcycler480, Roche).
A mutation identified after sequencing is shown on the right.



Codons 12 and 13



Mutation KRAS exon 2



c.35G>A
p.Gly12Asp

CONCLUSION

We show that analysis of MSI, KRAS and BRAF is feasible in surgical specimens of colorectal cancer after VCS. This procedure can be an alternative to immediate formalin fixation.

After this evaluation, this procedure has been implemented in our centre, as it avoids the use of formalin in the surgical theatre.

As this system allows to deliver unfixed material to the pathology laboratory, it can also be implemented when it is mandatory to frozen part of the tumour, as it is recommended for various tumour types.