Macrocryosectioning and complete sampling of the prostate in a potential multiorgan donor candidate

The incidence of prostate cancer overall in male donors is 3.1%.¹ There are no standardised procedures for its identification.² In general, the procedure is identical to that followed by urologists to investigate men with abnormalities in prostate-specific antigen, in digital rectal examination and in transrectal ultrasound—that is, multiple prostate biopsy specimens are taken.

The biopsy specimens can be processed with a fast technique, which yields permanent sections and diagnosis in 3 h.³ The alternative is frozen-section examination, the same technique used for intraoperative consultation. The processing time with the latter depends on the number of biopsy specimens and can be as short as 30–45 min. As with the biopsy procedure in general, cancer is not always detected, even though it may be present in the unsampled prostate. Anecdotally, in a recent case presented to one of our group, cancer was not found in the frozen biopsy specimens. The prostate was then removed and sent to a northern Italian centre. Cancer was eventually seen after an extensive frozen-section examination of several tissue samples.

The complete sampling procedure with the whole-mount technique—macrosections—is a standard approach adopted in several institutions, including ours, to examine formalin-fixed radical prostatectomy specimens with clinically detected cancer.⁴

In this short report, we describe our experience on macrocryosectioning and complete sampling of the prostate from a potential multiorgan donor candidate. It is not within the scope of the report to discuss whether the man whose prostate was removed was a suitable donor candidate based on his age and clinical history.

Case presentation
Clinical history

The case described here concerns a man aged 82 years. His history included a clinical diagnosis of prostate cancer made a few years earlier for which he was put under continuous androgen ablation therapy. There was no histopathological proof of cancer. His recent clinical history included an extensive spontaneous brain haemorrhage. He was considered a potential multiorgan donor candidate. The prostate was removed and delivered fresh within 10 min to the Pathological Anatomy Unit, United Hospitals and Polytechnic University of the Marche Region, Ancona, Italy. His serum prostate-specific antigen level was undetectable and digital rectal examination and transrectal ultrasound were negative for suspicious nodules.

Macroscopic examination, sectioning and processing of the fresh prostate

The radical prostatectomy specimen, which included the seminal vesicles, weighed 26 g and measured 3.5×3×2.8 cm. The external surface was not inked because there was no interest in surgical margin status. The apex and base were removed after a shave approach. The prostate was then step-sectioned with an 8 cm long microtome blade at 4 mm intervals perpendicular to the long axis (apical–basal) of the gland—that is, serial coronal sections,

parallel to an initial en-face section of the apex, were taken. Particular attention was paid to maintaining a constant thickness. Five slices were obtained.

The slices were placed on the cut-up table for inspection. The cut surface was white and homogeneous and compact in appearance. Suspicious areas were not seen. The slices were identified in a consecutive manner with numbers, starting from the most apical section. The first four en-face sections were processed as single sections—that is, macrosections. The other three en-face sections were subdivided into two halves, the right and left parts.

Each slice or part of a slice was placed directly on a pre-frozen tissue holder (a specimen disc) that had been previously covered with an optimal cutting temperature compound. Specimen discs of 4 cm in diameter (fig 1) were used to freeze simultaneously all the pieces in the chamber of a separate cryostat (Microm HM 500, Laborgeräte GmbH, Walldorf, Germany). This was kept at a temperature of –30°C. The specimens took approximately 5 min to become hard in consistency.

Sections of tissue 5 µm in thickness were cut and mounted on slides at least 76 mm long and 50 mm wide. The slides were stained with toluidine blue, cleared in xylene, dehydrated in graded alcohols and mounted using coverslips of a size similar to that of the slides.

Table 1 reports the approximate time for macroscopic examination, sectioning and processing.

After all the histological sections were examined under the microscope and the diagnosis was rendered to the clinician, all the material was de-frozen, fixed in formalin for 12 h and processed to obtain permanent sections as described previously.⁴ No attempt was made to further divide the tissue pieces.

Figure 1 Specimen disc used to freeze the tissue slices.

Table 1 Processing time (reporting time not included)	
	Time (min)
Examination of the uncut prostate	3
Slicing of the entire prostate, including examination of the cut surface	7
Freezing	<5
Sectioning, staining and mounting	~45
Total	~60

Frozen-section examination

The size and shape of the sections on the slides exactly corresponded to those of the frozen tissue slices. This means that the sections contained the entire tissue present in the cut surface of the frozen material.

The quality of the tissue preservation in the sections was excellent (fig 2). The details of the epithelial and stroma components were such that the nuclear features were preserved. The boundary of the so-called prostate capsule and the scant extraprostatic soft tissue were easily identifiable as such. There were very few ridges.

Cancer was identified in the peripheral zone of the left side of the prostate in two consecutive macrosections. Cancer showed regressive changes due to endocrine treatment similar to those described in routinely processed specimens (fig 3).⁵ Gleason grading was not feasible. Its largest diameter, measured on the surface of the slide, was 3 mm (fig 2). Cancer was localised to the prostate (ie, pT2a, according to the 2002 tumour, node, metastasis revision). The non-neoplastic ducts and acini were diffusely atrophic. Scattered foci of chronic inflammation were present in all prostate zones.

Comparison with permanent sections

Findings from the H&E-stained sections derived from fixed tissue exactly corresponded to those from the toluidine blue-stained sections obtained from frozen material (figs 4–6). In particular,

Figure 2 Toluidine blue-stained macrosection. The dotted area corresponds to the focus of cancer. The prostate slice from which this section was obtained is shown in fig 4.

Figure 3 Adenocarcinoma with regressive changes due to androgen manipulation. The nuclear details of the epithelial and stroma components are preserved.

cancer location and features corresponded to those seen in the frozen sections (fig 6).

Discussion

Our case presentation shows that an entire prostate can be successfully and rapidly examined from the histological point of view with macrocryosections.

The main advantages with the approach followed in this case are that the quality of the tissue is preserved and that the examination can be completed in a reasonably short time (table 1). The time from receiving the specimen to rendering the diagnosis to the clinician is approximately 1 h.

The requirements are those usually needed in routine frozen-section consultation—with

cryostats in good condition, including a low temperature in the chamber and a blade with a razor-like edge. The assistance of a skilled technician, like the one we had with us in the tissue-processing period, is necessary.

The major disadvantage is that a certain amount of tissue is lost when the first sections are discarded until complete ones are obtained. We did not calculate the amount of tissue discarded. However, our impression was that it was greater than that discarded when routinely processed material is cut with a microtome.

The limitation of the present description is that our experience is limited to a single case, even though this was successfully examined.

There was a previous short report on the feasibility of macrocryosectioning of the prostate.⁶ In that study, the authors reported their experience in 13 radical prostatectomies with clinically detected cancer. Only a single entire prostate slice per case was processed. They used a technique similar to ours. Comparison with permanent sections was not made.

Our report has some similarities with a recently published study, in which the frozen-section technique of the prostate was used in the setting of multiorgan donor cancer screening.⁷ In that study, however, only 50% of each specimen was analysed and macrosectioning was not performed.

Few papers have been published on the use of frozen-section examination of radical prostatectomy specimens with clinically detected cancer. In such cases, the intraoperative approach is used for the management of intraoperatively detected palpable tumour lesions during nerve-sparing scheduled radical prostatectomy and surgical margins, whereas the pathological examination of the main bulk of the prostate is performed on permanent sections.^{8–11}

In conclusion, macrocryosectioning of the prostate is feasible. However, more experience is needed with additional cases.

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Plasma DNA restoration for PCR applications

Plasma DNA is a valuable source of a wide range of molecular indicators that relate to the general condition and stage of cancer patients. However, continual patient monitoring and/or long-term studies require the use of archived plasma samples, frequently yielding a poor quality DNA upon storage. A restoration treatment was developed for plasma DNA that includes a pre-PCR incubation with *Taq* polymerase, dNTPs and 1× PCR buffer that improves the efficiency of subsequent PCRs. Pre-PCR treatment of isolated DNA resulted in superior PCR efficiency and sensitivity of detection both on a fresh plasma DNA isolate and on the plasma DNA isolates from 12 long-term archived plasma samples from lung cancer patients. This procedure allowed the recovery of a group of plasma samples considered otherwise useless. It is concluded that the quality of archived plasma DNA can be remarkably improved by subjecting the samples to a pre-PCR restoration treatment.

Human blood plasma from both healthy and sick individuals contains variable quantities of nucleic acids. The majority of the mechanisms proposed for its release from cells are related to cell death by necrosis or apoptosis, though active release has also been suggested.^{1,2} Patients suffering from different diseases where there is a clear increase in cell death have comparatively higher plasma DNA levels than healthy individuals.^{3,4} This phenomenon

Figure 4 Slice of prostate after de-freezing and fixation in formalin.

Figure 5 H&E-stained macrosection obtained after tissue fixation. (see also figs 2 and 4 for comparison).

Figure 6 Adenocarcinoma as seen in the permanent section (see fig 3 for comparison).