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Proposal of a Punch Biopsy Protocol as a Pre-requisite for the Establishment of a Tissue Bank from Resected Esophageal Tumors

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Abstract With the development of tissue banking, a need for homogeneous methods of collection, processing, and storage of tissue has emerged. We describe the implementation of a biological bank in a high-volume, tertiary care University referral center for esophageal cancer surgery. We also propose an original punch biopsy technique of the surgical specimen. The method proved to be simple, reproducible, and not expensive. Unified standards for specimen collection are necessary to improve results of specimen-based diagnostic testing and research in surgical oncology.

Keywords Esophageal carcinoma · Punch biopsy · Tumor bank · Tissue bank · Surgical oncology

Introduction

Biological tissue banks are important in clinical practice and in research. The increasing interest of research groups in accessing such banks requires more standardized and simplified procedures to handle and collect specimen. Nowadays, only a few Centers can afford to invest in personnel specifically trained and dedicated to these procedures, and many academic institutions cannot even gather enough resources for creating such banks.

Standardized sampling, process and storage of biological materials are the critical factors that determine the quality of the outcome. A simplified procedure not only can reduce costs but can also improve quality by reducing individual and process-induced errors. Lower costs means that more Centers (even not academic) with limited resources can create their own biological tissue bank, thereby increasing the number of involved institutions and the volume of patients. A less expensive first phase sample collection may facilitate the creation of larger regional/national database, and move resources to the research phase. Tumor banks are now well-established in some countries [1, 2]. Central tissue banks have also been successfully established for the purpose of conducting international clinical trials [3, 4].

The incidence of esophageal carcinoma has significantly increased during the past three decades. Despite advances in surgical technique, the overall 5-year survival rates remain low. Patients with Barrett's esophagus have a 30-fold higher risk of developing an esophageal adenocarcinoma, which is the most common histological subtype of esophageal carcinoma diagnosed in the Western countries. Current therapies for esophageal carcinoma are not uniform, and no randomised controlled studies have conclusively proven the effectiveness of neoadjuvant chemo-radiotherapy in improving the long-term survival [5, 6]. In our center, more than 50 patients per year undergo surgery for esophageal carcinoma. A bank of tissue and biological samples is justified by the number of cases and by the need to intensify the research

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efforts for the prediction of tumor response to neo-adjuvant therapy.

We report a standardised, low-cost method to collect and store data and biological samples from patients with esophageal carcinoma, to be used for the purpose of high-quality research studies.

Materials and Methods

During October 2008 the storage procedures were assessed in a small-scale study that evaluated personnel and budget needs, quality and quantity of collected material, workflow, definition of roles and responsibilities, and networking among the groups involved in the research activity.

From November 2008 to October 2009, after formal approval by the Ethical Committee, ASL Milano Due (Protocol no. 74452), all patients referred to our hospital for esophageal carcinoma and listed for surgical intervention were offered enrollment in this protocol.

Informed Consent

Each patient was fully informed by the attending surgeon about her/his disease and about the currently available therapies. The importance of the study was also explained. If the patient agreed to participate, she/he signed the informed consent form and was enrolled in the study.

Case Report Form

Clinical data from each patient were collected and stored both on paper and electronic media using a specifically designed case report form (Fig. 1). This database, archived at the same time of sample collection, was made available to all physicians involved in the post-surgical treatment of the patient.

Sampling and Storage of Biological Fluids

Gastric juice, saliva, urines, and blood were collected from each patient. Gastric juice and saliva samples were collected at the time of upper gastrointestinal endoscopy and stored in criotubes at −80°. Urine samples were stored in a refrigerator until centrifugation at 3,000 rpm for 10 min. Supernatant was then stored at −80° in 50 ml vials. Blood, plasma, buffy coat were sampled in the fasting patient the day of surgery and collected in EDTA vials. Samples were stored in a refrigerator until centrifugation at 2,000 rpm for 10 min. Plasma was then stored at −80° in 2 ml criotubes. Each EDTA vial yields two samples, 2 ml of plasma and a few drops of buffy coat.

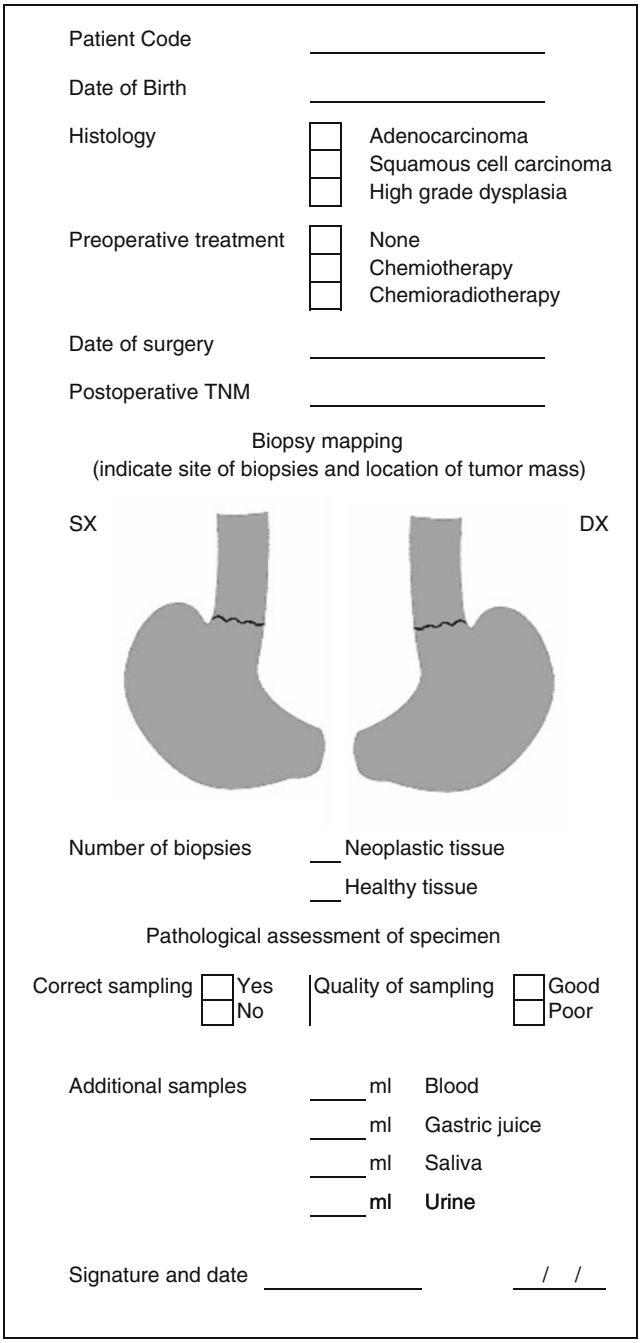

Fig. 1 The case report form used in the study

Biopsy and Handling of the Surgical Specimen

Fresh samples of tissue were immediately collected from the fresh surgical specimen. The specimen was carefully opened and washed with saline. Three biopsies of macroscopically healthy tissue and 1-2 biopsies of peripheral pathological tissue were performed using a punch device (HS Biopsy Punch, HS Hospital Service, Aprilia, Italy) and collecting 5 mm/diameter cylindrical, full-thickness tissue samples. These samples were stored in separate Eppendorf

vials, submerged for 3 h in RNAlater (RNAlater® Tissue Collection) and finally stored at −80°. The punch was positioned perpendicular to the longitudinal axis of the esophagus, and twisted into the wall to extract the specimen. The operation was first performed on healthy tissue to avoid contamination by tumoral cells. Subsequent samples were taken from neoplastic tissue avoiding necrotic areas (Fig. 2). The tumoral sample was marked with India ink on the endoluminal wall surface, and a similar mark was applied on the external biopsy area, to help correct identification by the pathologist (Fig. 3). Proper sampling procedure and depth of the lesion can be further assessed by pathological examination of a segment of the cylinder after formalin fixation. The final pathological report included a formal assessment of the sampling procedure based on identification of the ink mark and further sample analysis. The remaining gross surgical specimen was fixed with formalin and properly catalogued and stored.

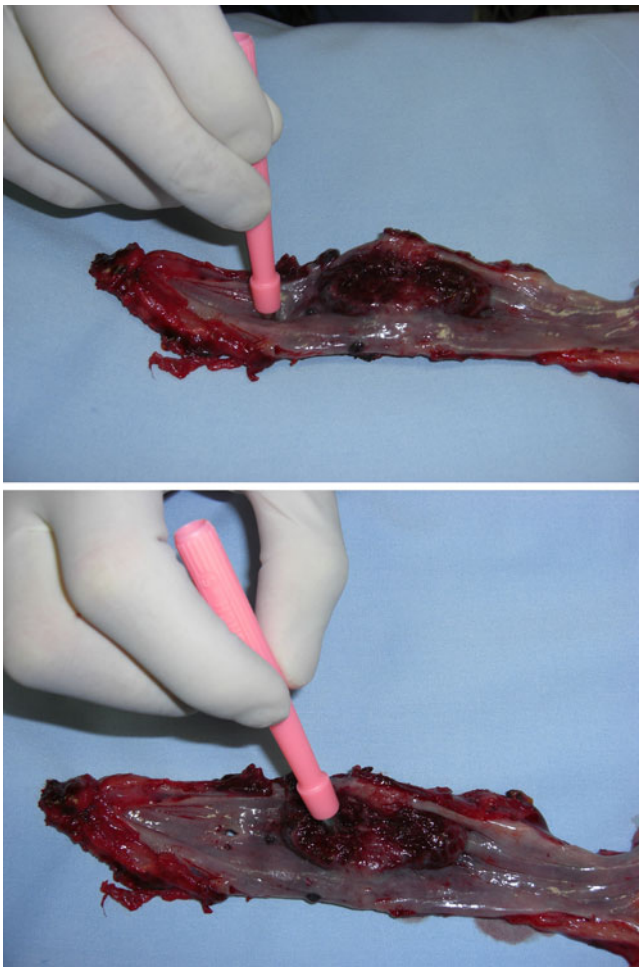

Fig. 2 The punch biopsy device is positioned perpendicular to the longitudinal axis of the esophagus and twisted first into the normal wall and then on the neoplastic tissue

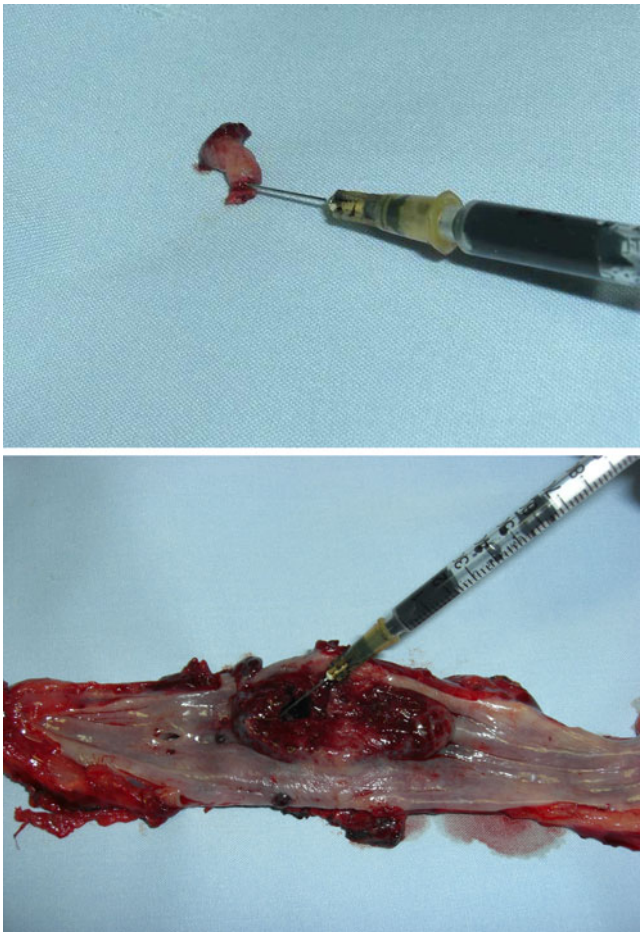

Fig. 3 The endoluminal side of the tumoral sample and the borders of the biopsy area are marked with Indian ink

Results

Over a 12-month period, 53 patients with carcinoma of the esophagus or cardia were enrolled in the study. One board-certified staff surgeon and one surgical resident were involved in sample collection, treatment and storage after a one-month training period. Samples from each patient were taken and stored as described. For each patient, a Case Report Form both on paper and on a electronic database was used to describe the samples. All the samples of saliva, gastric juice, blood and urine were treated and stored within 30 min from collection. Tissue collection was simple, easy and cheap. Tissue punching took an average of 5 min, and the sample was immediately put into RNAlater. Random biopsy examination confirmed the correctness of the sampling procedure. The biopsy protocol did not alter the pathological staging of the disease in any of the cases. All samples were easily stored after indexing. The cost of consumables for each patient was about 20 Euros. The digital archive allowed a close and easy coooperation between the involved hospital units by allowing a real-time, accurate monitoring of the patients' data.

Discussion

The progress in translational cancer research has led to an increase in the number and analytical power of investigational techniques. This has in turn increased the demand for high-quality tissue and biological samples. Genomic and proteomic research in the field of esophageal carcinoma is quickly growing with the aim to identify the predictors for response to chemoradiation therapy [7, 8]. In recent years, many biological tissue banks have been established. An important issue remains the variability arising from different collection methods. Variability of sampling techniques, treatment and storage may in fact impair the reproducibility of research studies. Harmonisation of the sampling process is indeed a critical step to improve the bank inventoires.

Several variables, both clinical and not, are involved in the collection of samples from surgical specimen.

Aim of this study was to reduce the non-clinical variables affecting the sampling of tissues or biological fluids and their storage. There is a consensus among biobanks to keep this time-lag as short as possible, with a "gold standard" interval set at 30 min. We have described our in-house protocol for the collection of fresh tissues. The reduction of the personnel and of the number of steps between sampling and storage of the surgical specimens also reduces the processing-phase variables that may affect sample eterogeneity and quality. An improved efficiency in the use of time, human and budget resources also occurs when the personnel involved in sample collection is informed and trained in the processing and storage techniques used in later phases. Our in-house collection protocol was entirely managed by two attending physicians. They were properly trained in the various sample processing techniques which were found to be simple, not expensive and not time-consuming, during a one-month testing period. All the biological fluids were processed and frozen at −80° within 30 min, thus overcoming the transfer, process and storage problems common to blood, plasma and serum collection. To avoid compromising the operative specimen by means of scissors or scalpels, we used an original punch biopsy technique that allows to obtain good-sized wall-to-mucosa samples of the tumor itself and adjacent tissue, and is very easy to perform on fresh surgical specimen. Tissue punch biopsy sampling is fast (to minimise RNA and protein degradation), does not require additional staff or special equipment (liquid nitrogen) for immediate sample processing, does not influence the pathological staging of the disease, does not require further histological confirmation nor great storage room. RNAlater allows indefinite storage and protection of RNA eliminating the need for immediate freezing.

Conclusions

Validated sampling procedures of surgical specimens after esophagectomy from carcinoma are not reported in the literature. Standard procedures, on the contrary, already exist for collecting healthy and neoplastic tissue from prostate and breast. We recommend a standardized protocol for collecting, processing and storing biological specimen from patients undergoing esophagectomy for carcinoma. The procedure we have described is simple, effective, easily reproducible, and not expensive. These features make the procedure affordable to academic and non-academic institutions interested to participate in research networks. The punch biopsy method we have described can also be used for sampling surgical specimen of other digestive tract tumors.

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