

TUMOUR METASTASIS AND DISSEMINATION DURING LAPAROSCOPIC SURGERY

Thesis submitted for the degree of Doctor of Philosophy in the University of Adelaide

by

Susan J. Neuhaus, MBBS (Adel)

The work described was performed within the

Department of Surgery of the University of Adelaide

and the

Royal Adelaide Centre for Endoscopic Surgery, Royal Adelaide Hospital

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from: Schaeff B, Paolucci V, Thomopoulos J. Port site recurrences after

laparoscopic surgery: A review. Dig Surg 1998;15:124-134.

ABSTRACT

Recent applications of laparoscopy to the resection of abdominal and thoracic malignancy have been followed by a burgeoning literature which describes cases of metastatic involvement of laparoscopic port sites, not only in patients with advanced tumours but in patients with early stage carcinoma, and even in patients following laparoscopic procedures during which tumours were not disturbed. The development of a port site metastasis in a patient following laparoscopic tumour resection with curative intent or the 'upstaging' of tumour stage, constitutes a failure of treatment.

Experimental studies incorporating bench top and large animal models have confirmed that tumour cells are redistributed to port sites during laparoscopic surgery directly from contaminated instruments, or indirectly in the insufflation gas. Of particular concern, a large number of experimental studies have demonstrated an increase in tumour implantation and metastasis to wounds following laparoscopic as compared to laparotomy techniques. Previous work by the Royal Adelaide Centre for Endoscopic Surgery suggests that the addition of a pneumoperitoneum may increase the rate of tumour implantation five-fold. Of pivotal importance is the question of what contribution the laparoscopic environment plays in the process of tumour dissemination and whether these effects can be modulated.

This thesis utilised an established small animal model to investigate the aetiology of port site metastases and the efficacy of preventive strategies in reducing tumour implantation following laparoscopy.