

Anniversary Issue Contribution

Thrombosis and cancer: A personal view

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During our Ph.D. studies at the Laboratory of Marc Verstraete, under the guidance of Jos Vermeylen (Leuven University), Giovanni de Gaetano and myself went to visit some US laboratories in 1972, including that of Eugene Clifton at Sloan Kettering in New York. This investigator was indeed one of the pioneers to propose the two-way association between thrombosis and cancer.

This association, described one century earlier by Armand Trousseau in tumors of the gastro-intestinal tract (1), had not been further explored, but warranted both experimental and clinical attention, as Clifton's work had shown in the 1960s (2–6).

Back to Italy in 1973, we were asked to open a new laboratory on haemostasis and thrombosis in Milan at Mario Negri Institute, a non-profit research institution well known at that time for its experimental studies in the field of cancer chemotherapy. We decided therefore to match the experience in the physiopathology of haemostasis and thrombosis – gained during our MD studies at Rome Catholic University with Raffaello Breda and Bruno Bizzi, two pioneers of coagulation studies in Italy, and extended during our five years spent at the Leuven Laboratory – with the experience of Mario Negri Institute investigators in the oncological field.

We started to work there at two levels: cell biology, looking with Luciano Morasca at the procoagulant/fibrinolytic activities of tumors cells (7–9) and animal models, with Andreina Poggi, looking at changes in the haemostatic system of the host during the development of some experimental tumors (10, 11) and on the treatment of murine tumors with antithrombotic drugs (12–14).

A cancer procoagulant

Many studies were focussed on a new procoagulant activity specifically confined to the malignant phenotype, the so-called "Cancer Procoagulant". This factor appeared to be similar to the one described in rabbit V₂ carcinoma by Stuart Gordon's group in Denver, Colorado, and was found by our group in several warfarin-sensitive murine tumors (9, 16, 17) and in human melanoma

cells (18), as well as in different human tumors (19). It was also the main procoagulant activity of acute promyelocytic leukaemia, and a sensitive marker of both disease activity and response to chemotherapy and ATRA treatment (20–22). Cancer procoagulant had been purified from human amniocorial or several malignant tissues as a cysteine protease with a peculiar clotting mechanism by Anna Falanga in Gordon's Laboratory. Despite the effort of several laboratories in the past 20 years, such an important tumor cell procoagulant and malignancy marker, for a number of reasons including technical problems, has not received so far a complete molecular and genetic characterization, which remains open to future research.

Studies in experimental models of tumor growth led us to the definition of the type of thrombocytopenia and anaemia associated with the growth of the primary site in some murine tumors (10, 11) and to the discovery that warfarin could exert a peculiar antimetastatic effect in some models, possibly due to the inhibition of cancer procoagulant, characterized as a novel vitamin K-dependent factor (16, 17, 24). Other studies in mice, with Nicola Semeraro, Alberto Mantovani, Mario Colucci and Roberto Lorenzet, led us to realize how important tumor/host interactions were in the expression of a "physiological" extravascular procoagulant such as tissue factor (TF) with consequent fibrin deposition at the tumor/host interface (25, 26). These studies pioneered a large series of experiments on inflammation, cancer and host antitumor defence mechanisms.

Despite the progress our group and few others had accomplished in the field, the association of thrombosis and cancer was mainly considered, till the end of the 1970s, the concern of some "exoteric" investigators and the presentations proposed at haemostasis and thrombosis meetings were regularly ranked as "other" or "miscellaneous". As they were not included in any major category, they were usually accepted as posters, to be presented during the less important sessions. A honourable exception was the 1979 Congress of the International Society on Thrombosis and Haemostasis (ISTH) in London, where the late Arthur Bloom organized a symposium on "Malignancy and Haemostasis", I was asked to chair together with John Davidson (27). The increasing interest of our group into "cellular aspects of haemos-

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tasis”, was also reflected by the “Bizzozzero Symposium” organized together with Peter Mannucci in Bergamo (1982) on the occasion of the 28th ISTH SubCommittee meeting.

In 1983, within the ISTH, we succeeded in establishing a new SSC SubCommittee devoted to “Malignancy and Haemostasis”. Its first chairman was Yale Nemerson. I took the chair in the subsequent years (1985–1988), followed then by Graham Jamieson, Fred Rickles, Mark Levine, Ajay Kakkar, Anna Falanga and Martin Prinz (2007). These SubCommittee meetings gradually moved from 20–30 attendees to over 300.

From experimental models to clinical trials

The real switch was determined by the growing interest of clinical oncologists in the association between thrombosis and cancer, along two main lines:

- The association of thrombosis with cancer treatment, not only surgery, but chemotherapy and hormone therapy as well. This concern has lately been extended to the risk of thrombosis associated with anti-angiogenic therapy (28–31).
- The observation of idiopathic or unprovoked venous thrombosis as a predictor of the clinical manifestation of an occult cancer (32–34).

These two clinical issues have prompted new research and inspired experimental work in the last few years. Meanwhile, the work of the SubCommittee had led to the establishment, in collaboration with Leo Zacharski, of a Registry of Clinical Trials of Antithrombotic Drugs in cancer patients, first published in 1989 (35) and updated in 1993 (36). The Registry clearly showed a very messy situation in a period when, in other fields such as the cardiovascular one, the application of modern clinical trial methodology had allowed to reach important goals for prevention and treatment of ischaemic heart disease (37–38).

Indeed, at the experimental level, evidence had been collected to suggest that modification of the host's haemostatic system by different pharmacological approaches could lead to changes in the degree and speed of tumor and metastasis growth (12–14). The data could be schematically summarized as follows: Whatever antithrombotic drug or condition, such as antiplatelet antibodies or dysfunctional platelets, would reduce blood cell stickiness and/or increase blood fluidity, would also delay and reduce the trapping of tumor cells within the lungs of the host by diverting cancer cells towards the spleen or the liver, to be destroyed there by the reticulo-endothelial system. As a result, the subsequent growth of lung metastasis would be significantly delayed or inhibited (12, 14, 39, 40). These data did not find initially any translation into clinical application, and for many years a striking lack of appropriate clinical trials was reflected in the SSC Registry.

One of the major difficulties to prove the effectiveness of anticoagulants in cancer patients was that the use of antivitamin K drugs – remarkably effective in some animal models (13, 16, 24) – required laboratory monitoring and induced serious haemorrhagic risk in patients with cancer, who are often thrombocytopenic due to chemotherapy and/or radiotherapy.

The development of low-molecular-weight heparins (LMWH) in the prevention/treatment of thrombosis in cancer patients, has lately made available a more patient-friendly antithrombotic treatment in these patients. The favourable experience with LMWH opened indeed the way to new trials with heparins to modify the natural history of several tumors (42, 43). This happened during the last few years and is still going on. From preliminary data available in the first trials (44–47) it would appear that LMWH treatment could delay metastasis growth in different tumors, but only if the tumor is still at an early stage. This finding, confirmed by trials with different design and treatment schedules (44–47), may be explained by the following hypothesis: LMWH would act on the dissemination of tumor cells by anti-adhesive/anti-inflammatory properties (different at least in part from their anticoagulant activity); these properties would enable LMWH to prevent the adhesion of tumor cells to the vascular wall and their extravasation to form metastatic foci. Once metastases have been formed, however, LMWH would be unable to prevent their growth. For this reason only tumors at a relatively early stage, i.e. with still a significant burden of circulating tumor cells, would respond to the inhibitory or delaying effects of LMWH. This hypothesis is supported by recent *in vitro* data showing the ability of some LMWH to prevent cell-cell interactions, relevant for the adhesion of cancer cells to the vascular wall and subsequent extravasation (48, 49). Old experimental work in mice had already shown the best time window of treatment with heparin to obtain an anti-metastatic activity: This effect was indeed only observed when tumor cells were injected intravenously in mice pretreated with heparin (40).

Conclusions

The relationships between thrombosis and cancer are a paradigmatic example of the useful cross-talk between experimental data and clinical results: many years after the reports on beneficial heparin effects in experimental tumor models (4, 5), recent clinical results (44–47) prompted us to go back to experimental systems, to find out the best conditions for heparin to favourably modify the natural history of some human tumors. Presently, these studies need to carefully consider the mechanism of heparin's action in relation to both tissue factor expression by cancer and host cells and the angiogenic response, two areas of significant progress in recent years (50–52). Although studies on the association between thrombosis and cancer are no more confined to few laboratories, the great number of open questions at experimental and clinical levels will certainly keep the scientific community busy for many years to come.

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