JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **16** (2005) 1213– 1219

In-vivo short- and long-term evaluation of the interaction material-blood

ANTONIETTA M. GATTI[1], S. MONTANARI[2,*], A. GAMBARELLI[1], F. CAPITANI[1], R. SALVATORI[1]
[1]*Laboratory of Biomaterials, University of Modena and ReggioEmilia, Italy*
[2]*Nanodiagnostics srl, Via E. Fermi, 1/L, 41057 San Vito, Modena, Italy*
E-mail: gatti@unimo.it

Through an innovative electron microscopy technique, thrombi and fibrotic tissue taken from 14 explanted vena cava filters were observed. Twelve cases showed the presence of micro-and nano-sized inorganic, non biodegradable nor biocompatible particles which did not belong to the metal the device was made of and which could be the sole cause or, more likely, a pre-existing cause for thrombosis. In two cases, those debris activated immunological reactions typical of a foreign body. The presence of inorganic particles in the blood was never detected before and their effects on human health are hardly known. Their thrombogenicity should be added to the Virchow's Triad as a fourth factor and could be the explanation to many of the cases of pulmonary embolism where no thrombotic focus could be demonstrated.

1. Introduction

Checking the *in-vivo* interaction between a material or a device and the blood has always been a problem hard to solve. Direct measurement techniques to evaluate the response of the blood components to the presence of a foreign body introduce themselves an interaction between the device used for the test and the blood, thus modifying a number of parameters and preventing to obtain the direct, unimpaired view of the interactive phenomenon.

The analysis of explanted vena cava filters can offer such a possibility without influencing the system.

Those filters, usually implanted in the lumen of the inferior vena cava, are the most common mechanical device for the prevention of pulmonary thromboembolism and their use is growing more and more widespread, especially in the USA. In 1999, a new model of vena cava filter was made available in Europe, that can be used as a permanent device or be retrieved, though not in all cases, even after a comparatively long time. The observation of the filters explanted allows the direct study of thrombi captured in the human venous circulation and the punctual interaction between blood and device.

Trough traditional histologic methods, captured thrombi and the tissues formed around the filter can be observed, while an innovative Environmental Scanning Electron Microscopy technique allows the search for micro-and nano-sized foreign bodies inside both thrombi and tissues, and the assessment of their chemical elemental composition by means of Energy Dispersive Spectroscopy. The study of micro- and nano-sized foreign bodies inside human tissues and their behaviour is a fundamental part of the new discipline called "Nanopathology".

Aim of the present work was the analysis of the tissues found in the explanted filters as specific of the device/blood interaction from a morphological, immunological and nanopathological point of view.

Any discussion or consideration on the indications to the use of caval filters or their clinical effectiveness is outside the scope of this work.

2. Materials and methods

The vena-cava filters examined (ALN Implants Chirurgicaux, France) are composed of nine AISI-316 L stainless steel, 0.3 mm diameter, wires gathered together inside an ogival capsule made of the same material, from where they branch out, forming a conical skeleton. Each of the shorter 6 of the 9 prongs that make up the device has a distal hook that penetrates the venous wall and serves to keep the filter anchored in place, while the longer three are meant as stabilizers to keep the device lined up with the vascular axis, and have no hooks. One of the long prongs has a small ring at its distal end. The overall height of the device is 5 cm and, being it elastic, its diameter adjusts itself to the section of the vessel up to 32 mm.

14 ALN filters were implanted either as a preventive means before surgery in patients who were deemed at risk of developing a deep-vein thrombosis (DVT) with a consequent pulmonary embolism (PE), or to prevent PE episodes in potentially relapsing patients.

*Author to whom all correspondence should be addressed.

TABLE I

	Days of implant	Clinical indication	CD8	CD68	Size	Chemistry
1	18	Prophylaxis for loco-regional thrombolysis	neg	pos	0.3–8 μm	C, Ca, O—C, Ca, O, S, Si, Na—C, Si, Al, O, Mg, K, S, Fe—C, Fe, O, S, Si, Cu, Ca—C, Si, O, Na, Ca, K—C, Fe, Cr, O, S, Si, P, Al—C, Ni, O, S, Na—C, O, Si, Al, K, S, Mg—C, W, S, O, P, Na, C—C, Bi, Cl, S, O, P, Na, Cu—C, Zr, O, S, Na—C, O, Fe, S, Na—C, Si, O, S, Al, K, Na—C, Fe, Cr, S, O, Ni, Si, Na—C, O, W, Co, S, P, Na, Ca, Cl—C, O, Ca, Mg, Si, Al, S, Na—C, Si, O, Al, S, Na, Ca—C, Ag, O, S, P, Ca, Mg—C, Si, O, Al, Mg, S, K, Fe, Na—C, Al, Fe, O, Cr, S, Si, Cu
2	19	Prophylaxis for hip-joint prosthetic substitution	pos	pos	0.4—24 μm	C, Al, O, Si, Ca, Na, Fe—C, O, Si, Al, Ca, Zn, S—O, C, Na, Fe, K, Cr—O, C, Cr, Fe, Si, S, Na—Si, Mg, O, C—Si, O, C—C, O—C, S, O, Ba, Na—C, Ca, O, S, Na, P—Fe, C, Cr, Ni, O, S, Si, P, Na, Cu—C, Fe, O, S, Mn—C, Fe, O, Sb, S, P, Si, Na—C, Ca, O, Si, Mg, Al, S, K, Fe, P, Na
3	90	Floating thrombus	neg	pos	/	/
4	96	Prophylaxis for cancer surgery	pos	pos	/	/
5	98	Prophylaxis for abdominal surgery	pos	neg	0.1—10 μm	C, Fe, O, P, Ca, Si, S, Al—C, Bi, Cl, O, Ca—C.O, S, Si, Ba, Ca, P, Fe—C, Fe, O, Cr, Ni, S, Si, Ca
6	106	Femur fracture with DVT	neg	neg	0.1—30 m	C, Fe, S, O, Cu, Ca, Si, Ti—C, O, S, Ba—Si, C, O, Al, S, Mg, K, Fe—C, Ca, O, Si, Al, Mg, P, S, Fe—C, O, Si, Al, S—C, Fe, Cr, S, O, Ni—C, O, Fe, S, Al, P, Cu, Ca, Si, Ti, Sn, Cr, Na—Al, C, O, Si, Na, Ca—C, W, O, S, P, Fe—C, O, S, Ba, P, Al, Si, Ca, Na, Fe—C, Fe, Cr, O, S, Ni, P, Si, Al, Ca, Cu—C, Si, O, S, P—C, Si, O, Al, K, S, P, Na—C, O, Sb, S—C, Si, O, Al, S, Fe, Mg, Ca, K, Ti—C, Fe, O, S, Si—C, Si, O, S, Ca—C, Al, O, S, Ca, Fe, Si—C, Si, Mg, O, Fe, S—C, O, Pb, Si, Al, Cr, Fe, Mg, Ca—C, O, S, Ba, Ca, Na
7	107	DVT in viral hepatitis	pos	neg	0.4—10 μm	C, Ca, O, S, Na—C, Fe, O, Cr, S, Si, Mg, Ca, Na—C, O, Si, Al, Mg, Fe, S, Na, K—C, S, O, Ba—C, Al, Fe, Cr, O, Ni, S, Si—Na, C, O, P, Cl—C, Fe, O, Sb, S, Na, P, Si, Mn—Si, C, O, Al, Ti, K, Ca, Fe—C, Cl, Na, O, Al, Si, P, S—C, O, Na, P, Cl, Si, S, Al—Cl, Na, C, O—C, O, Na, Si, P, Al, Ca, Cl, S, Fe, Mg—C, Ca, O, S, P—C, Si, O, Na, Al, Ca, Mg, S, K—C, Fe, Cr, O, Ni, S, Si, Ca—C, Cr, O, S—C, Sb, O, S—C, Zn, O, Ca, P, S—C, Cu, Cl, O, S, Ca, Si, K, Fe
8	130	Patella fracture with DVT	neg	neg	0.1—10 μm	C, Ca, O, S, Ba, Al—C, Fe, O, S, Al, Si, Ca—C, Ca, O, S, Al, Si—C, O, S, Ag, Si, Al, Ca—C, O, Ti, S, Ca, Al, Na—C, Fe, Cr, O, Ni, S, Si—C, Ag, O, S.Fe, Cr, Si, P, Al, Mg, Cl, Ca, Ni, Cu—C, Fe, O, Cr, N, Ni, Mo, P, Si, Ca, Na, Cu—C, O, Fe, P, Na, S, Cr, Si—Ba, S, Fe, C, O, P, Cr, Na, Ni—C, Zr, O, S—C, Si, O,

(*continued on next page*)

TABLE I (*continued.*)

	Days of implant	Clinical indication	CD8	CD68	Size	Chemistry
						Na, Ca, Al, Mg, S, K, Ti—C, Ca, O, Si, Al, S, K, Mg, Fe—C, Fe, Ti, O, S, Ca—C, Ca, O, S—C, S, Na, O
9	166	Rectum cancer with DVT	neg	pos	0.1–10 μm	C, Ba, S, O, Ca—C, Fe, O, Cr.Ni, S, Ca–Fe, C, Cr, O, Ni, Ca, S, Si
10	170	Prophylaxis for DVT	pos	neg	0.5–6 μm	C, Fe, Cr, O, Ni, S, Ca, Si—C, Zr, O, S, Ca—C, O, S, Ca
11	172	Tibial fracture with DVT	pos	pos	0.1–5 μm	C, Fe, O, Cr, S, Cl, Ni, P—Si, C, Al, Cl, O, Fe—C, Cl, K, S, O—C, Fe, O, P, S, Cl
12	235	PE with DVT	pos	neg	0.2–10 μm	C, Fe, O, S, Ca, Cr, P, Ni, Si—Ca, C, O, S, Cl, P, Mg, Na—C, O, S, Cl, Ca, Bi, P, Na, Mg—C, Ag, O, S, Ca, P, Mg—C, Au, Cl, S, O, Ca, Ag, Na, Si, Mg, Cu, Fe—Si, C, Al, K, O, Ca, S, Mg, Na
13	293	Head trauma with DVT	neg	pos	0.2–1 μm	C, Ti, O, S, Ba, Cl, Ca—C, Fe, O, Cr, Cl, S, Ni, Si
14	384	PE with DVT	neg	neg	0.2–20 μm	C, O, S, Bi, Ca, P, Si, Na, Fe, Cu—C, Fe, O, P, S, Ca, Na, Cr—C, Fe, O, Cr, S, Ni, P, Si, Na, Ca—Al, C, O, S, Fe, Ca, Ti, Cr, Ni, P—C, O, Al, Si, K, S, Na, Ca, Mg, P, Fe—C, Bi, Cl, O, S, Na, Ca, Cu—C, Si, O, Mg, Ca, Na, S, K—C, Fe, O, P, Ca, S, Na—C, Ca, O, S, P—C, Fe, Cr, O, S, Ca, Ni—P, C, Ca, Zn, O—C, O, Au, Ca, Al—C, Ca, O, Al, P, S—Zn, C, O, P, Ca, Mg—Si, C, O, Na, Ca, Al, Mg, K, Ba, Cl, P, S

The filters were sent to the Laboratory by different Italian hospitals after implant periods ranging from 18 to 384 days.

As soon as the filters were explanted, they were put in a jar containing formalin solution and were delivered as such to the Laboratory.

There, the jars were opened, the filters extracted and observed under optical stereo-microscope (Nikon-Japan) at low-magnification to look for evidence of corrosion. Then the tissues and the thrombi adhering to the filters were removed by means of a lancet and tweezers. The location inside the filter whence each specimen was taken was noted.

The samples were immersed in alcohol 70%, dehydrated in ascending concentration of alcohols and embedded in paraffin. 7-micron-thick sections were cut (Leitz microtome, Germany) from the paraffin blocks. Some were deposited on glass for histology and immuno-chemistry observations, others were laid on a rectangular sheet (4 × 2 mm) of tri-acetate for electron-microscopy observations. A full series of sections was stained with hematoxylin-eosin and observed under optical microscope (Leitz, Germany). Other complete series of sections were treated for the immunological studies aimed at verifying the CD3, CD8, CD9 and CD68 marker expressions.

The sections laid on the tri-acetate sheets were de-paraffined with drops of xylol and 98% alcohol, then pasted with a carbon disc on a 4 mm-diameter aluminium stub and observed under an Environmental Scanning Electron Microscope (ESEM) (ESEM-Quanta, FEI Company, The Netherlands) with a technique that has already been described in literature [1]. That instrument works on non-electron conductive samples, either at medium vacuum or at room condition.

The ESEM used was equipped with an Energy Dispersive System (EDS) that permits to obtain the elemental chemical analysis of the foreign bodies found in the samples, if any.

3. Results

No corrosion phenomena were visible in any of the filters, including the ones that had stayed a long time implanted, as inspected at low-magnification under optical stereo-microscope.

All filters showed the presence of fibrino-haematic tissue, in some cases vascularized, with fibrocytes and fibroblasts adhering to the metal structure and most

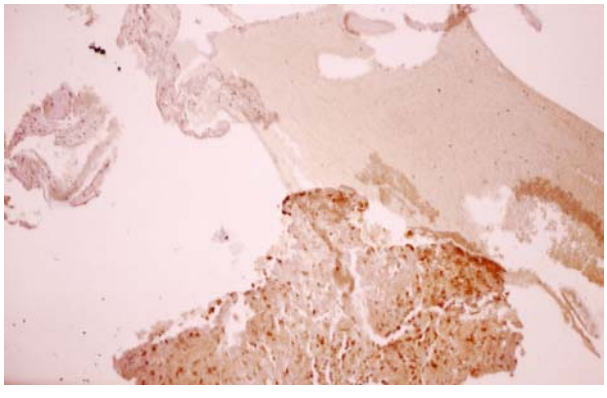


Figure 1 Case 2 Fresh thrombus with isolated lymphocytes (20×).

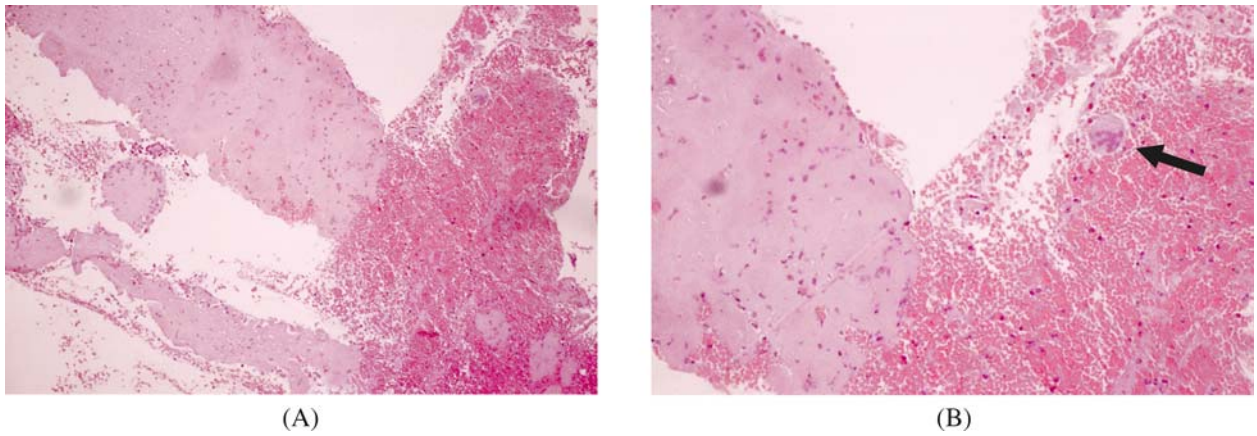


Figure 2 (A) Case 8 Fresh thrombus in a more advanced state of organization than the one of Fig. 1. Fibrous tissue, macrophages and a small foreign-body giant cell are present (10×). (B) Case 8 Detail of the former image. The giant cell is indicated by the arrow (20×).

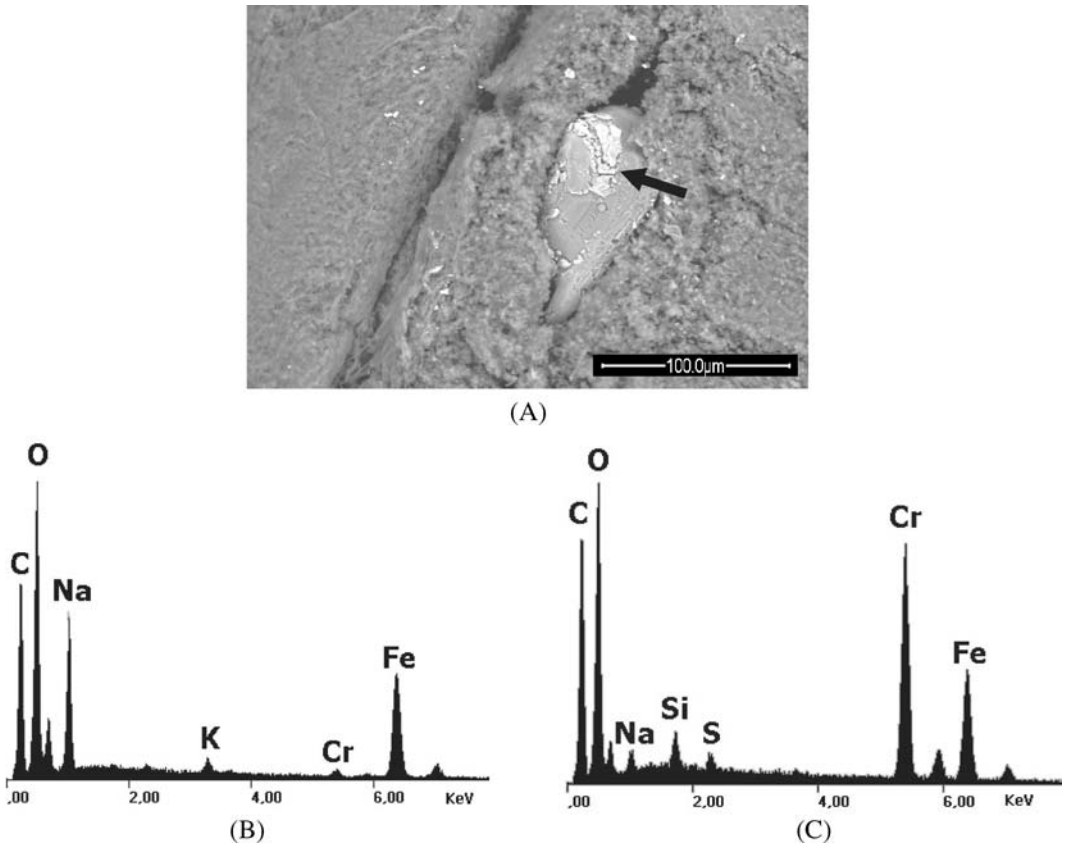
of them contained more or less organized thrombotic material imprisoned inside the vertex of the cone.

Table I displays the most significant histo-chemical (CD8 and CD68) and the nanopathological results obtained from the 14 specimens examined. What is meant here with the word "nanopathological" is the content of inorganic micro-and nano-particles.

Fig. 1 (case 2) shows a fresh thrombus with isolated lymphocytes, while Fig. 2 (case 8) shows again a fresh thrombus whose organization is more advanced than the one of the former. In Fig. 2(A), an older, fully organized, thrombus is shown, where fibrotic tissue and macrophages are visible. At higher magnification (Fig. 2(B)), a small giant cell due to the presence of a foreign body can be seen.

Fig. 3(A) is an ESEM pictures showing the same sample as the one in Fig. 1 Here, the foreign body is clearly visible and its chemistry is shown in the EDS spectrum of Fig. 3(B) and (C). A macrophage can be seen phagocytizing the foreign body.

From the histo-chemical point of view, the most interesting samples look those regarding cases 10 and 11, respectively for the expression of CD8 immunoreactive T-lymphocytes (Fig. 4), and for the expression of CD68-positive cells (Fig. 5). Fig. 6(A)



(A) (B) (C)

Figure 3 (A) Case 2 ESEM image of a section of the thrombus containing a large particle (arrow) where two different chemical compositions are observed ((B) and (C)). (B) Case 2 EDS spectrum of debris (A) containing Na, Fe and Cr. (C) Case 2 EDS spectrum of the lighter area of the debris of (A) containing Cr, Fe and Si.

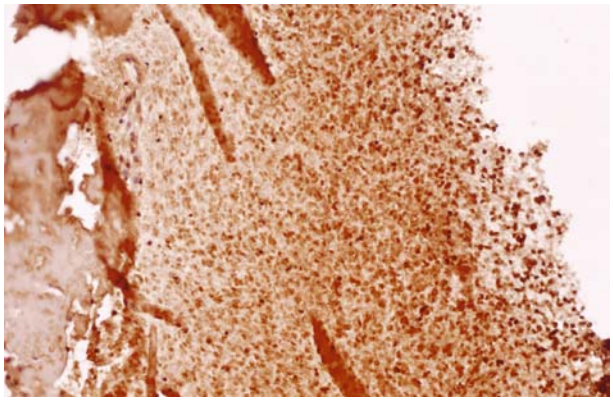


Figure 4 Case 10 CD 8+ Isolated CD8 immuno-reactive lymphocytes *(black cells at the edge of the tissue)* (20×).

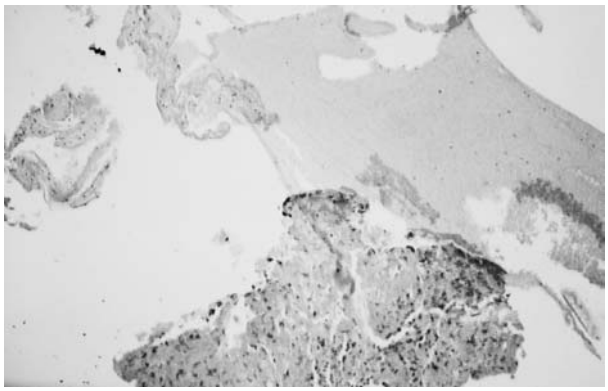


Figure 5 Case 11 CD 68+ The appearance of CD68-immuno-reactive macrophages accompanies the organization of a thrombus (10×).

shows a Fe particle (Fig. 6(B) is its relevant EDS spectrum).

Samples 3 and 4, as observed through the ESEM, did not show the presence of any foreign bodies.

Sample 1 was particularly interesting from the nanopathological point of view, as the thrombus contained micro-and nano-sized foreign bodies composed of as many as 6 different chemical compositions (Co–W, Al–Si, Si, Fe, Ag–S, Fe–Cr–Ni). Fig. 7(A) shows a particle of W detected in sample 1 with its EDS spectrum (Fig. 7(B)). The morphology of this debris looks rather like a cluster of nanoparticles.

Another interesting finding is the one relative to case 7, where clustered nano-particles of Al were found (Fig. 8(A) and (B)).

4. Discussion

The presence and quantity of thrombotic material found in the filters did not seem to depend on the time the device had stayed implanted in the organism.

Twelve of the fourteen cases examined showed that micro- and nano-sized inorganic foreign bodies were present inside the thrombi captured by the filters. That does not necessarily mean that the two "particle-free" specimens did not contain any foreign body. Our technique, in fact, does not allow the detection of the elements lighter than Beryllium including the latter, and C and O that could make up a foreign body are covered by C and O present in great quantity in all biological tissues. In none of the cases we examined, the elements found belonged either to the human organism or to the composition of the filter. Fe, Cr and Ni, the components of AISI 316 L but also of other stainless steels, were found only occasionally alloyed together and no filter showed any trace of corrosion that could explain that presence. It must be observed that, in any case, the inorganic elements detected were not in their ionic form but found as actual particles, whose sizes ranged from 20 μ to 50 nm.

Such presences in the blood were never described before our technique became available.

It has been demonstrated that foreign bodies the size of 100 nm, once inhaled, can leave the alveoli and enter the blood circulation in 60 s [2]. In the cases taken into consideration in this work, also much larger than 100-nanometer particles were detected in the blood. Whether they are the cause of the thromboses or they are just mechanically trapped by the blood during its pathologic coagulation is uncertain, but it is not unreasonable to suppose that a foreign body can be thrombogenic, especially, as has always been the case in this study, when those foreign bodies are neither biodegradable nor biocompatible, and being not biocompatible means, in the vast majority of cases, being also thrombogenic [3].

The size and composition of the debris found is rather typical of industrial pollution, in particular of high-temperature processes involving inorganic materials, such as foundry, incinerator or engine fumes. Thus, the likeliest way of entry into the organism is inhalation. Nevertheless, other mechanisms may not be ruled out, and among them, the ingestion of polluted food.

All patients were considered to be at risk of developing a deep-vein thrombosis (DVT) or, in some cases, were already diagnosed to be affected by that disease. This is one of reasons that make us suspect that the thrombi detected or, at least, part of them, embolized from the DVT foci. Some of them, though, could have been originated by inorganic particulate circulating in the blood and become actually thrombogenic once the organism has been stimulated by particular, well known, conditions like, for example, surgery, bone fractures, cancer or a relatively long stay in bed. It is very unlikely that the filter itself is thrombogenic. In that hypothetical event, in fact, the thrombus started inside the filter should have grown flow-wise and only a very small part of it would be in a section of the device where the flow is slowed down or altogether absent, [4] which has never been the case in our specimens, where the thrombi were always totally contained within the geometric limits of the device.

The only reaction found between filter and its environment is the growth of a modest quantity of fibrosis, in some instances with some vasculature, developed between the venous intima and the metal structure, especially at the distal ring placed at the tip of one of the three long prongs.

The immune reactions observed only in two cases (phagocytosis, CD8 and CD68) are likely to be triggered by the presence of foreign bodies, as suggested by the macrophage attacking a particle. According to a long-established pathology principle, no pulmonary

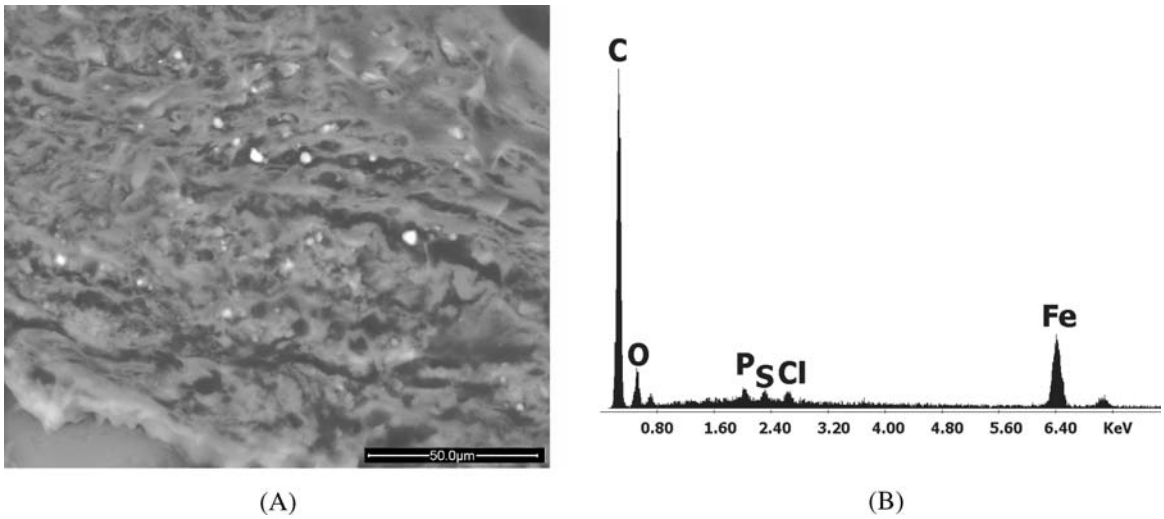


Figure 6 (A) Case 11 ESEM image of a section of the thrombus containing micro-and nano-particles disseminated in the thrombus. (B) Case 11 EDS spectrum of the Fe nanoparticles from the debris.

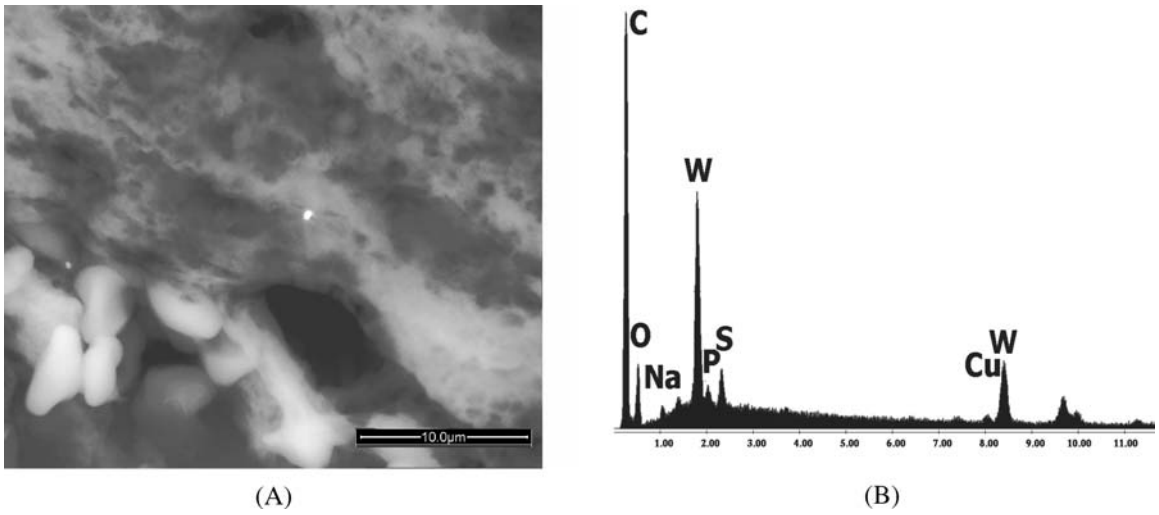


Figure 7 (A) Case 1 ESEM image of a thrombus containing a cluster of nanoparticles. (B) Case 1 EDS spectrum of a nanoparticle containing W and Co.

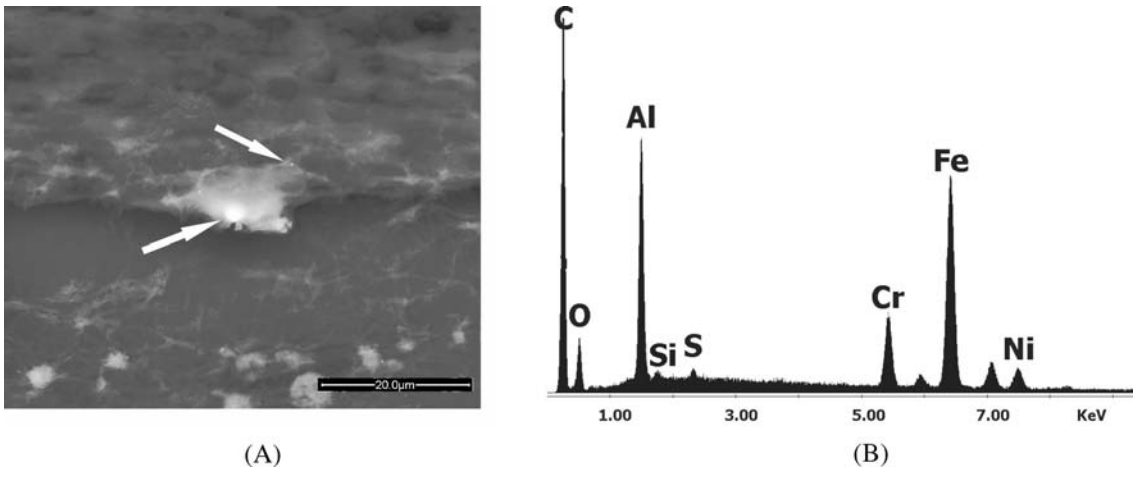


Figure 8 (A) Case 7 ESEM image of a thrombus containing a round-shaped micro-and a nano-particle. (B) Case 7 EDS spectrum of the round-shaped particle of (A), containing Al, Fe, Cr and Ni.

embolism occurs without a DVT [5], but it is a well known fact in daily medical practice that in some, far from being rare, instances no focus can be demonstrated through either a venography or a colour-Doppler investigation, despite the occurrence of recurrent PE episodes.

Conclusions

That particulate air pollutants are responsible for or, at least, have some form of relationship with the onset of cardiovascular and pulmonary diseases has been amply demonstrated [6, 7] and is supported by clinical evidence, though much remains to be explained as to the pathomechanisms they follow.

The original technique we developed allowed us to observe directly and for the first time the presence of micro- and nano-sized, not introduced artificially, "foreign bodies" in the blood. That debris is very likely to be responsible for triggering immunogenic reactions and for the formation of thrombi *in vivo*.

Thrombi have been observed adhering to the ALN vena-cava filter, but they look to be due to the capability of capture of that device and not to its thrombogenicity, which cannot be proved, at least in our series. No final evidence exists, either, that the device brings about immunogenic reactions.

The inorganic micro- and nano-sized particulate matter found in the thrombi seems to be thrombogenic and responsible for the formation of at least part of the clots observed.

A number of the many pulmonary embolism episodes observed worldwide are classified as idiopathic, since no focus can be identified nor an explanation regarding their origin is possible according to the so-called Virchow's Triad, that states that pathological clotting of the blood in the vessels occurs when the blood flow is somehow obstructed or slowed down, when the vascular endothelium is damaged or when the chemistry of the blood is disturbed or incompetent. In many instances, it is a combination of the three factors that is responsible for the thrombosis, as one single factor may not always be enough.

The presence of particulate matter in the blood, added to the Triad as a fourth factor, may explain in part or totally the not infrequent cases when none of those classical three causes exist, yet a pulmonary thromboembolism occurs.

A thing that would certainly deserve a deeper investigation, something we could not do as we did not have access to the patients involved in this work, was the research for symptoms that may have gone unnoticed or unexplained, due to the possible further toxic effects caused by the chemistry of the particles detected (for example Co, W, Sb, Pb, etc.).

The awareness of the phenomenon and being able to identify and characterize those particles can help understand their pathomechanism and contrive effective means of prevention.

Acknowledgments

The authors are indebted to Professor Giuseppe Barbolini, histopathologist of the University of Modena for the suggestions he offered. This research was supported by the European Community: Project "The role of micro and nanoparticles in inducing biomaterial pathologies" QLK4-2002-147.

References

1. 1 A. M. GATTI and S. MONTANARI, in "Handbook of Nanostructured Biomaterials and Their Application in Nanobiotechnology", edited by H.S. Nalwa, (ASP, Stevenson Ranch, Cal. 2005), vol. 2, p. 347.
2. A. NEMMAR, P. H. M. HOET, B. VANQUICKENBORNE, *et al*., *Circ.* **105**(4) (2002) 411.
3. J. BLACK, *Biomat.* **5** (1984) 11.
4. S. MONTANARI, in "Malattia Tromboembolica Venosa e Filtri Cavali", edited by C. Rabbia and G. Emanuelli (Edizioni Minerva Medica, Turin, 2000) 90.
5. R. VIRCHOW, *Arch. Pathol. Anat. (Berlin)* **10** (1856) 225.
6. J. M. SAMET, F. DOMINICI, F. C. CURIERO, *et al*., *N. Engl. J. Med.* **343** (2000) 1742.
7. A. PETERS, A. DORING, H.E. WICHMANN, *et al*., *Lancet* **349** (1997) 1582.

Received 30 June
and accepted 18 July 2005