In search of biological and radiological markers of aneurysm instability

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Abdominal aortic aneurysms (AAA) are a common disorder affecting 4 % of the population over the age of 65. The risk of rupture is currently estimated according to diameter, but while some large aneurysms may remain intact, it isn't rare for small aneurysms to rupture. Teams at the CHU hospital (Department of Cardiovascular Surgery, Department of Nuclear Medicine) and the University of Liège (Laboratory of Connective Tissues Biology, GIGA) are on the trail of markers that can predict the instability of abdominal aneurysms.

For most of us, the term aneurysm is generally associated with the notion of rupture and usually a fatal outcome for the patient. This type of death is particularly shocking owing to its violent nature, because the disease's slow evolution prior to rupture is most often asymptomatic. The distinction between the formation of the aneurysm and its rupture isn't always easy to discern or understood by non-specialists.
An aneurysm is a localised and irreversible dilation of an artery wall. The appearance of this disorder is favoured by certain factors such as high blood pressure or smoking, among other things. The rupture of an aneurysm, and therefore the artery wall, causes significant and often uncontrollable bleeding. Depending on the location and the type of blood vessel concerned, this rupture can lead to the patient's rapid death. In 95 % of cases, the aneurysms are located in the abdominal artery. "This isn't a rare condition since it affects 4 % of the population over the age of 65. Furthermore, it is inevitable that the number of aneurysms detected will continue to increase, with the gradually aging population", explains Professor Natzi Sakalihasan, from the Department of Cardiovascular Surgery at Liège's CHU, lead by Jean-Olivier Defraigne. Aortic aneurysms can be detected using a simple ultrasound scan. However, owing to its often asymptomatic nature, it is often not detected before the rupture occurs. "The rupture is fatal in 60 to 85% of cases, most often even before the patient has been admitted to hospital", Professor Sakalihasan stresses.

### Identifying dangerous aneurysms

In humans, the normal diameter of the abdominal aorta is 18 to 22 millimetres. "When its diameter reaches 30 millimetres or more, the dilation is considered as pathological", Natzi Sakalihasan points out. Patients are therefore recommended to attend regular check-ups to monitor the evolution of the expansion of their aneurysm. In current medical practice, surgery is recommended when the aneurysm's diameter has reached 55 millimetres, based on the principle that the more it expands, the higher the risk of rupture. "But not all large aneurysms rupture, while small ones sometimes do", stresses Natzi Sakalihasan. "What's more, in older patients, surgery can sometimes do more harm than good." Taking these arguments into account, Professor Sakalihasan has spent the past 25 years or more studying the definition of criteria that would allow us to define abnormal growth and, above all, assess the risk of rupture. "We are endeavouring to find the means to identify potentially..."
dangerous aneurysms", specifies the professor. To this end, he is working in close collaboration with GIGA's Laboratory of Connective Tissues Biology (LBTC) at the University of Liège, led by Professor Alain Colige and the Department of Nuclear Medicine led by Professor Roland Hustinx.

Connective tissues constitute the majority of our body mass and are particularly involved in supporting, protecting and feeding organs. They are made up of cells dispersed in a network of molecules forming fibres, such as elastin and collagen, thus creating an extracellular "matrix". "Big blood vessels, such as the aorta, are essentially comprised of two layers of connective tissue of a different composition. This setup is responsible for the mechanical resistance and elasticity of the arterial walls, two fundamental properties allowing them to adapt to the stresses and deformations associated with the blood flowing through the vessels each time the heart beats", points out Professor Colige.

An efficient but expensive scanner

During previous studies, Natzi Sakalihasan and Alain Colige's teams examined, among other things, the proteins in the extracellular matrix of the aortic walls: elastin and collagen, which are responsible respectively for elasticity and the mechanical resistance of connective tissue. "In simple terms, we could say that the deterioration of the elastin causes the appearance of the aneurysm and that its rupture is generally associated with the destruction of the collagen", summarises Natzi Sakalihasan. The researchers have already shown a correlation between the instability of an aneurysm and the presence of MMP-9 and -2, enzymes that participate in the deterioration of elastin and collagens.

Another study carried out in 2002 by the teams at Liège's CHU and CHC, revealed that recourse to the PET-CT (positron emission tomography - computed tomography) medical imaging method was efficient in detecting aneurysms that are likely to rupture. "This type of scanner uses a radioactive marked glucose analogue as a tracer to spot areas of high metabolic activity, such as inflamed tissue", explains Natzi Sakalihasan. "Since abdominal aortic aneurysms can be considered as a chronic inflammatory disease, we thought of using a PET-CT scan to examine patients", he points out. The researchers were thus able to observe that aneurysms with a high metabolic activity ran a greater risk of rupture. This method could thus allow doctors to decide whether surgery is necessary, regardless of the size of the aneurysm. But this solution isn't accessible to everyone: "Not all hospitals are equipped with a PET-CT and the examination is quite expensive", Natzi Sakalihasan reminds us.

Looking for markers circulating in the blood

Within the framework of a new study recently published in the Journal of Nuclear Medicine (1), Natzi Sakalihasan, Roland Hustinx and Alain Colige focused on the cells and molecules associated with areas of high metabolic activity in abdominal aortic aneurysms. The objective of this work was to identify circulating biomarkers that would convey the presence of an unstable aneurysm. "In the present study", explains Alain Colige, "we had the opportunity to compare human samples from patients with a negative or positive result from the PET-CT. In these latter subjects, we analysed components of the aortic wall in both areas of high and low metabolic activity". Work on these different types of samples would allow researchers to pinpoint the cells and molecules that were likely to cause the rupture of the aneurysm.

At LBTC, Audrey Courtois, a doctoral student supervised by Betty Nusgens, analysed the variation in the abundance of RNA messengers in order to determine if there was a correlation between the results obtained by the imaging technique (PET-CT) and the presence of specific molecules in the samples of aortic wall taken...
from the areas of high metabolic activity in the aneurysm. "We wanted to see if some molecules were expressed more in these areas and if their presence could serve as a marker for the risk level of the aneurysm", Alain Colige continues. The analysis of the samples thus enabled researchers to confirm the presence of large infiltrates of inflammatory cells in places where metabolic activity was particularly high. At the same time, it allowed them to also identify the enzymes produced by these cells, responsible for the deterioration of the aortic wall.

**Systematic screening in Liège soon**

The analyses of Alain Colige's team have also shown that the expression of certain genes linked to the remodelling and maintenance of the aortic wall is different in aneurysmal areas with significant metabolic activity. Furthermore, changes in the structure and composition of the blood vessel's wall have been revealed. "For instance, in these areas, there is loss of smooth muscle cells, responsible for the blood vessel's mechanical activity. Moreover, part of the collagen present in this area isn't typical of vascular walls but rather of cartilage!", the researchers stress. While the exact meaning of these changes is still unknown today, the authors of this study strongly suspect that they contribute to the weakening of the aortic wall before rupture.

As regards the markers indicating the level of risk of abdominal aortic aneurysms, the scientists have pinpointed potential candidates. This work will be the subject of a future publication...

In parallel to this research work, Professor Natzi Sakalihasan will soon launch a systematic screening campaign for AAA among the population of Liège, with the collaboration of the Department of Cardiovascular Surgery at
Liège's CHU and the Aneurysmal Pathology Foundation (APF) which is funding this initiative. A mobile medical team will soon be visiting the communes of the Province of Liège to roll it out.