ITACAT: IMPACT OF THROMBUS ANALYSIS IN STROKE PATIENTS IN CATALONIA

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1. Summary

This project was conceived to improve both the diagnosis and treatment of stroke. Our group is made up mostly of stroke expert neurologists from different hospitals in Catalonia who attend stroke patients on a daily basis. In recent years, removal of the thrombus with a catheter (thrombectomy) has radically improved the course of stroke. In addition, it has allowed us to dispose of the thrombus that had caused the stroke. We believe that it could be useful to role-reverse the thrombus, from being responsible for ruining a patient's life to being useful for improving stroke diagnosis and treatment. The general objectives of the project are summarized thus: to find whether analysis of the thrombus allowed us to learn where it had formed (to know the cause of the stroke); to see if the thrombi most resistant to extraction had a different composition; to analyze the proteins that form the thrombus; and to assess if we could directly determine its composition with a brain scanner.

To carry out the study we relied on the solidarity of the patients who had an acute stroke and were admitted to our hospitals (Vall d'Hebron Hospital, Germans Trias i Pujol Hospital and Bellvitge University Hospital). Participants in the study were invited patients (with informed consent) who had had the thrombus extracted and analyzed, after being attended by the emergency services and after receiving appropriate treatment for the stroke. In addition, their heart rhythm was recorded for several weeks to look for hidden arrhythmias. Conventional outpatient visits were carried out, initiating the necessary treatments in each case to prevent complications.

In short, the methodology used to determine the cause of the stroke was to compare the thrombi formed in the heart with those formed in the carotid artery. To see if the thrombi that were the most difficult to remove were different from the rest, we compared the composition of the thrombi that required more than one thrombectomy attempt (attempting to remove the thrombus with a catheter) versus those that were easily removed with a single attempt. Advanced proteomic analysis was performed to identify and quantify the proteins involved in thrombus formation processes. For the analysis of the thrombus using brain scan images, an algorithm was created by artificial intelligence (AI) techniques. Thus, the images were quickly and automatically reviewed to find out whether the thrombus existed in the brain and to know its composition.

The thrombus was analyzed using preparation, fixation and conservation techniques by expert personnel from the Pathological Anatomy Service of the Vall d'Hebron Hospital. Both conventional (hematoxylin and eosin) and advanced (immunohistochemical) techniques were used to determine the amount of red blood cells, platelets, fibrin, and leukocytes that each thrombus had.

2. Results

The results of the study showed that thrombi in the heart and carotid arteries are different. Heart thrombi have more platelets, while carotid thrombi have more leukocytes (specifically one type, TCD4 lymphocytes). This occurs in the context of an inflammatory process, which is more evident with the rupture of the carotid plaque than with the thrombus formed by deposition in the heart (most often due to an arrhythmia called atrial fibrillation). These results are relevant, since many studies that have evaluated the composition of the thrombus have not been able to detect differences in its origin. This is because they used conventional techniques to analyze the most basic elements (fibrin, red blood cells, etc.) In our study, we used more complex stains to analyze other types of cells, e.g. platelets, lymphocytes and macrophages, which allowed us to study this population extensively.

In addition, with this analysis we have verified that thrombi rich in platelets are harder than those with fewer platelets. This has to do with the organization of the thrombus, since platelets activate several factors that facilitate the formation of platelet networks that are wrapped in fibrin (a protein that hardens the thrombus). In addition, we have been able to replicate these results in a new technique, which had never been used before to analyze thrombi (flow cytometry), which makes us pioneers in this field. Protein analysis confirmed that thrombi are different in the heart and carotid arteries. It was also shown that, although the thrombus forms in one place or another, the protein composition can vary in each patient. For example, within the thrombi formed in the heart there are those with a composition more similar to the disease of the arteries and others related to muscle contraction (with proteins such as myosin-14, myosin-9, myosin-12A).

The automatic analysis of the brain scanner has made it possible to know the composition of the thrombus very quickly and automatically, since a special algorithm has been created to perform the analysis. Compared to conventional thrombus analysis techniques, this AI algorithm could detect thrombi with high erythrocyte composition with high diagnostic validity (77% sensitivity and 85% specificity).

3. Relevance and possible future implications

The results obtained are relevant since they help to improve both the diagnosis and the treatment of stroke. In cases in which the cause of the stroke is unknown, the study of the thrombus will enable us to know if it has a profile from the heart or from the carotid artery. Thus, with a thrombus profile from the heart, covert causes of embolism such as atrial fibrillation will be sought. In other cases, when the patient has diseases that increase the risk of embolisms of both the heart and the carotid artery, thrombus analysis will ascertain the real cause of the stroke.

Whether the thrombus is going to be easy or difficult to remove with thrombectomy is a key factor to choose the best catheter to remove it. However, this requires extracting part of the thrombus for analysis, which could delay the information. For this reason, it was proposed to use an alternative technique such as thrombus cytometry, which allows the thrombus to be analyzed very quickly (in minutes). But the thrombus can be analyzed even faster if the brain scan is checked by an AI algorithm to find out what type of stroke it is, thus making it possible to select the treatment that most benefits the patient.

The results of the protein analysis help to find the exact composition of the thrombus, which may allow more specific treatments to enhance thrombus rupture (thrombolysis), even personalizing the treatment to each thrombus and each patient (personalized medicine).

With the results obtained and the experience accumulated, the research group collaborates with other international networks to analyze thrombi and improve the prognosis of stroke patients.

4. Scientific bibliography

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