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PhD Thesis

"Evaluation of the safety, efficacy and histological changes of a new mixture of Glubran®2 + Ethanol + Lipiodol ® (G.E.L.) as embolic agent in neurovascular procedures – animal lab experience"

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Table of contents

Abstract

Purpose: The aim of this study was to investigate the degree of penetration, permanence of occlusion and vascular changes induced by a modified mixture of n-butyl cyanoacrylate (Glubran 2®), ethanol and Lipidol® (G.E.L.) in endovascular treatment of experimental aneurysms induced in swine.

Methods: Bilateral pouch aneurysms were created in the wall of the internal carotid artery in eight pigs. The sixteen aneurysms were treated with a mixture of G.E.L. in different dilution between components. Angiograms were obtained at the time of treatment and at 1, 4, and 16 weeks after treatment. According to the schedule experimental design, subjects were sacrificed at the time of treatment and 7-, 30-, and 90-days after embolization of experimentally induced aneurysms. The internal carotid artery and aneurysms were resected en bloc and fixed for histopathologic study.

Results: All the experimentally induced aneurysm were completely occluded by the used mixture and remained occluded for the study period without recanalization. Histopathologic studies revealed the presence of an acute inflammatory reaction of aneurysm wall even after 7 days post embolization with focal angionecrosis, followed by a chronic inflammation which induced a thickening of the wall. No inflammatory changes were observed in the host tissues at the periphery of the experimentally induced lesion.

Conclusions: The mixture used for embolization of aneurysms succeeded to induce a complete occlusion which remained stable until 90 days post-embolization. After seven days from embolization, the mixture polymerization was associated with an acute inflammatory response followed by a connective tissue proliferation which induced a thickening of aneurysm walls, without involvement of host tissue at the periphery of experimentally induced lesions.

Keywords: Angionecrosis, arteriovenous malformation, endovascular embolization, experimentally induced aneurysm, N-butyl cyanoacrylate.

Introduction

Medical glue was first used for embolization in 1975 which is extremely challenging, as the occlusion is permanent and virtually instantaneous. Therefore, glue (cyanoacrylate) embolization should be reserved for specialized centers. Cyanoacrylates and n-butyl 2-cyanoacrylate (NBCA) are intermediate-length cyanoacrylate adhesive and were the first product to be broadly used in medicine for closing cutaneous wounds; infused into the intravascular lumen can be toxic to neural tissues and vessel walls. Cyanoacrylate is mainly used to permanently occlude vascular abnormalities such as cerebral and spinal arteriovenous malformations [1] as well as in portal vein embolization, preoperative renal tumor embolization, embolization of endoleaks through endoprostheses and embolization for bleeding control.

The most widely used cyanoacrylate glue is Histoacryl® (B/Braun, Tuttlingen, Germany) (**Fig. 1a**), or N-butyl-2-cyanoacrylate, which is also available as Trufill® (Cordis, Miami Lakes, Florida) and has superseded isobutyl cyanoacrylate.

In recent years, attention has focused on the fact that Histoacryl® has no official recognition for endovascular use which is

considered off label. This situation raises major legal problems deriving from its improper use.

For this reason, in 2000 a new acrylic glue, Glubran 2® (GEM SRL, Viareggio, Italy) was first marketed. in which N-butyl-2-cyanoacrylate is combined with another monomer, metacryloxysulpholane (MS) (Fig. **1b**), to produce a more pliable polymer whose milder exothermic reaction (45°C) results in less inflammation and histotoxicity [2]. In fact, the longer the carboxylic side chain of the minor cyanoacrylate is, the less cytotoxic the resulting adhesive [3]. Metacryloxysulpholane also gives surgical Glubran 2 a significant anti-inflammatory effect. The long-term fate of Glubran 2® in the body is unknown. Based on this premise, the addition of MS to NBCA yielded a glue complying with European regulations and hence receiving CE certification for internal and endovascular use.

As these liquid monomers in isolatiare non-viscous, radiolucent, and rapidly polymerize, NBCA is generally mixed with lipiodol for visibility under fluoroscopic control [4–6]. It has been reported that the NBCA polymerization time can be regulated by adjusting the ratio of lipiodol to NBCA [7–9].

Further studies showed that the polymerization of cyanoacrylate or NBCA can be accelerated by the addition of ethanol [10]. Indeed Kawai et Al. (2012) discovered more recently that the addition of ethanol to the mixture of NBCA plus lipiodol had the effect of changing the configuration of NBCA polymerization. They evaluated increasing ratios of ethanol, investigated the properties of the mixture of NBCA with lipiodol plus ethanol and evaluated the feasibility of this mixture for aneurysm packing in a swine model. They found that with high ratios of ethanol, the mixture NLE (NBCA+Lipiodol+Ethanol) polymerization configuration acquired solid-like properties with potent occlusive ability and negligible adhesion to the microcatheter, suggesting its feasibility for packing of aneurysms.

Tanaka et al (2015) evaluated the feasibility of balloon-assisted packing with a mixture of N-butyl-2-cyanoacrylate (NBCA), Lipiodol, and Ethanol for wide-neck aneurysms.

Over the last years new embolic agents have been also developed with improved handling characteristics and AVMs penetration. Among these non-adhesive liquid embolic materials, so called gelling solutions, some are now largely used for endovascular treatments. Gelling solutions are composed of a polymer in a solvent.

The polymer solidifies in situ as the solvent is replaced by water. The first gelling solution, described by Taki in 1990 [12], was composed of ethylene-vinyl-alcohol copolymer (EVOH) suspended in dimethyl-sulphoxide (DMSO).

This non-adhesive liquid embolic material is easy injected through a microcatheter, when it comes into contact with an aqueous solution, like blood, its solvent dimethyl sulphoxide (DMSO) dissolves into the solution, and its main component ethylene vinyl alcohol copolymer (EVOH) precipitates and forms a spongy polymer cast [13]. Studies performed in animal models demonstrated that when used for endovascular embolization DMSO tends to be angiotoxic and neurotoxic [14]. Further studies demonstrated the neurotoxicity of DMSO when administered via the intracarotid route due to the high permeability to tissues and membranes [15]. The inflammatory reaction in the vessel wall is less pronounced than with cyanoacrylates and is largely confined to the blood vessels. There is no reaction (such as lymphocyte migration to perivascular areas) in the surrounding interstitium.

Although endovascular treatment of artero-venous malformations (AVM) with embolic agents, both adhesive and non-

adhesive, is nowadays largely used as standard of care in neurovascular interventions, likewise the same treatment cannot be applied for cerebral aneurysm as the long polymerization time of this embolic agents leads to high risk of uncontrolled migration and thus damage of healthy vessels and tissues.

None of the studies available in literature investigated the use of the mixture Glubran® 2 (n-butyl-cyanoacrylate + metacryloxysulfolane NBCA-MS) associated with Ethanol and Lipiodol as a new embolic agent for aneurysm endovascular treatment.

The aim of this study was to investigate the degree of penetration, permanence of occlusion and vascular changes induced by a modified mixture of n-butyl cyanoacrylate (Glubran 2®), Ethanol and Lipidol® mixture in experimental aneurysms induced in swine.

The primary endpoint is to assess the efficacy and the safety of the embolization treatment with the G.E.L. The secondary endpoint is to characterize, by histopathological analysis, the tissue response to this mixture.

Materials and Methods

Animals

In this preclinical study were used tissue samples from 9 healthy swine, six females and two males, weighing 50–70 kg.

Experimental design

The experiments were performed at the Biotechnologies Centre of A.O.R.N. “Antonio Cardarelli” Hospital (Napoli). Permission to conduct this experimental study was granted before starting of the study by the Institutional Committee on Research-Animal Care and the Italian Ministry of Health (Authorization n. 23654/19), in according with Italian Laws DL.gs 26/2014 and Directive 2010/63/EU on the protection of animals used for scientific purposes.

For each swine, two aneurysmal defects were created for a total of eighteen. Prior to surgery each animal was administered zoletil 50/50 + propofol + ketamine + sevorane (0.5ml kg +6mg/kg+10mg/kg + sev.2%) and butorphanol as pre-operative analgesic. General anaesthesia was induced with isoflurane gas via tracheal intubation.

Cardiac and pulmonary data were monitored throughout the procedures.

Creation of Aneurysm

Carotid aneurysms with wide necks were created by using the jugular vein. Briefly, the right and left carotid arteries and jugular veins were surgically exposed. A length of jugular vein approximately 3 cm long was removed and then sutured to an incision in the carotid artery, under interruption of carotid artery blood flow.

Each swine goes surgical exposure of the left carotid artery and the jugular vein to create an aneurysm. First, the jugular vein is removed, and a length of vein approximately 3 cm in length is removed. The resected piece of vein is sutured to the carotid artery on the same side as the incision to create an aneurysm model under interruption of the carotid artery. Every swine underwent surgical creation of the aneurysms, ranging in size from 5 to 30 mm, two aneurysms for each swine for a total of 18 aneurysms.

Subsequently a femoral access is performed that will allow to place a carrier catheter in one of the two carotid axes and will be provided with the use of a double microcatheter of which the first will

be used for the injection of the GEL mixture at the concentration established while the second microcatheter will have the characteristic of having a distal balloon that will allow the blocking of the flow at the aneurysm sac, thus preventing the uncontrolled migration of embolizing material from the sac itself. The animal will be placed in anti-aggregative therapy to avoid spontaneous thrombosis of the created bag.

In the first three days it was administered Tramadol 10 mg / kg orally in the drinking water and antibiotic therapy was continued for 7 days. Animals were checked daily for signs of adverse effects.

Immediately after surgery and at 7-, 30-, and 90-days post-surgery, two swine were sacrificed by anaesthetic overdose (isoflurane) and submitted to surgery to remove the experimentally induced aneurysms. The experimentally induced defects were excised in toto and immediately placed into 10% buffered formalin fixative.

Gel used for embolization

For embolization was used a mixture composed by Glubran 2 NBCA + MS (GEM Srl; Viareggio (LU) Italy) was withdrawn through a 21-gauge needle and mixed with contrast agent

Lipiodol (iodinated ethyl esters of the fatty - Guerbet) and ethanol (99.9 %, FUSO Pharmaceutical Industries, Ltd.). Glubran®2 was mixed with ethanol and Lipiodol in the ratio 2: 0.5: 1 (57% Glubran®2, 14% EtOH, 28%Lipiodol). This ratio was used because it yields a mixture that polymerizes in longer times that allow a controlled filling of the entire aneurysm.

It was prepared by first mixing ethanol and Lipiodol in a vail and was shaken by hand for 1 minute and then adding the NBCA+MS and shaken all for three minutes.

The mean long × short diameters ranged from 30 to 5 mm. The mean neck width ranged from 3 to 10 mm. The dome-to-neck ratio (long diameter/neck width) ranged from 1 to 3. There were no statistically significant differences among the aneurysms regarding aneurysm size or neck width.

The amount of the injected GEL mixture will be variable in relation to the size of the created aneurysm. We measured the volume of the aneurysm and estimated the total volume of GEL required. Packing of the aneurysmal model was attempted with different mixtures of GEL.

Endovascular procedure

In each swine, packing of the aneurysm was attempted few hours after the creation of the aneurysms. The embolization procedure was performed on the swine at an angiography facility (GE, USA) **(Fig. 2)**.

Activation clotting time of 200 s or more (normal range <120 s) was maintained by intravenous infusion of heparin (50 U/kg) prior the surgery and the embolization. Digital subtraction angiography, test injection, and the interventional procedure were conducted after adjusting the tube angle for optimal visualization of the lateral aspect of the aneurysm.

Carotid arteriography was conducted via a guiding catheter (Envoy 5Fr or 6Fr, Cerenovous, USA) placed in the target vessel within which a 0,017" microcatheter (SL10, Stryker, USA) was advanced to the aneurysm neck and inside the sac, using a 0.014-inch micro-guide wire (Transend EX; Boston Scientific, Natick, Massachusetts).

Subsequently a 4,35 Fr Remodeling Balloon catheter (Copernic, Balt, France) was advanced into the carotid artery and inflated at the neck of the aneurysm to a pressure of 6 atmospheres. Because the mean

diameter of the carotid arteries was 5.2 mm (range 4.4–5.9 mm), we used a 6 mm balloon catheter.

The gluing microcatheter is then purged with saline, to clear any residue of contrast material, and primed with 5 % glucose with a volume to match the catheter dead space (approximately 1.2 ml) to prevent polymerization of NBCA in the microcatheter.

After a new control of activated clotting time of >200 seconds (normal range, o 120 s), the carotid artery was occluded by balloon inflation. The mixture was slowly injected in small increments from the distal end of the aneurysm sac by use of a 3-ml syringe to avoid regurgitation in the microcatheter **(Fig. 3).**

Morphological studies

Histopathology

The formalin fixed experimentally induced aneurysms were routinely processed for paraffin embedding. Five-μm serial sections were dewaxed in xylene, rehydrated in a graded alcohol series and stained with hematoxylin and eosin (H-E) for routine examination, Masson's trichrome stain to evaluate connective tissue, Perls' Prussian

blue stain to detect hemosiderin deposits, and picrosirius red stain to determine the collagen types of contents. Slides were examined by two pathologists (F.P. and A.P.) in a blinded fashion.

Immunohistochemistry

For immunohistochemistry tissue sections were mounted on treated glass slides (Superfrost Plus; Menzel-Glaser, Germany). Sections were dewaxed in xylene and rehydrated through graded alcohols. Heat induced epitope retrieval with citrate buffer pH 6.0 was performed in a microwave oven. Immunohistochemical labelling was carried out manually with the Sequenza slide rack and cover-plate system (Shandon, Runcorn, UK). Non-specific peroxidase activity was blocked with Bloxall blocking solution (SP-6000; Vector Laboratories, CA, USA) Non-specific antigen binding was blocked by incubation with UltraVision Protein Block (TA-060-PBQ; Thermo Scientific, Cheshire, UK). A panel of primary antibodies was applied to serial sections and incubated overnight at 4°C. For macrophages an anti-Iba1 rabbit polyclonal serum (Wako, Neuss, Germany; diluted 1:300) was used, for T-lymphocytes an anti-CD3 rabbit polyclonal antibody (Dako, Glostrup, Denmark; diluted 1:200) and for B-lymphocyte an anti-

Cd79a mouse monoclonal antibody (Exbio, Praha, Czech Rep.; diluted 1:100). Antibody binding was detected by the Biotinylated horse anti-Mouse/Rabbit IgG antibody (H+L) R.T.U. (Vectors Laboratories, CA, USA), the Streptavidin-biotin-peroxidase kit (Vector Laboratories, CA, USA) and the 3,30-diamino-benzidine as chromogen (Vector Laboratories, CA, USA) as indicated by manufacturer's instructions. Stained slides were subsequently counterstained in haematoxylin for 50 seconds followed by a wash in tap water, dehydration in graded alcohols (70, 90, and 100%), and cleared with xylene. Sections were mounted in DPX (08600E; Surgipath Europe, UK). Substitution of the primary antibody with unrelated matched primary antibody was used to provide a negative control. Serial sections of a rat lymphnode were used as positive control.

Morphometrical studies

The aneurysm wall was submitted to a morphometric investigation. Bright field images were acquired at x40 magnification with a Leica Microsystem DFC490 digital camera mounted on Leica DMR microscope (Wetzlar, Germany). Counting was performed using a semiautomatic analysis system (LASV 4.3, Leica) on six 15,000 μm^2

random fields of three different areas of the aneurysm wall were examined. These sampled areas were used to evaluate the thickness of the aneurysm wall, the thickness of the connective tissue production, the new blood vessels count, and the composition of cell populations involved in the inflammatory reaction.

Statistical Analysis

Statistical analysis was performed using the statistical package SPSS Advanced Statistics 21.0 (SPSS Inc.,Chicago, IL, USA). ANOVA test was used to compare the different parameters observed at different time and when different CA glues were used. Post hoc analysis was made by Bonferroni Test. Statistical significance was based on a 5% (0.05) significance level.

Results

All the scheduled interventions have been completed and all the swine underwent aneurysm embolization. A total of 18 aneurysm have been taken into account.

- In **77,78%** of cases (14/18) aneurysms have been successfully and completely fulfilled by the mixture without immediate or delayed complications
- In **11,11%** of cases (1/18) aneurysms have been completely fulfilled but after balloon deflation embolic complication occurred as the mixture migrated in the parent artery
- In **5,56 %** of cases (1/18) the rupture of the balloon catheter occurred with consequent mixture migration in healthy territory.
- In **5,56 %** of cases (1/18) aneurysm has not been treated because the swine was suppressed before the treatment.

Looking at the swine the fate is the following:

- In **66,67%** of cases (6/9) swine survived after the procedure and underwent scheduled controls and sacrifice according to the protocol.
- In **11,11%** of cases (1/9) swine were successfully embolized but died 24 hrs. after the procedural probably due to ischemic complications
- In **22,22%** of cases (2/9) swine were suppressed immediatly after the procedure due to embolic or hemorrhagic complications.

Endovascular procedure

After initial injection of approximately 1-1.3 ml GEL, a test injection was performed. The mixture was slowly injected through the microcatheter under fluoroscopic control. When packing was achieved, the balloon catheter was deflated 5 minutes later, and digital angiography was performed to verify the occlusion rate. When packing was insufficient, an additional injection was attempted using the same mixture under balloon occlusion to reduce the aneurysmal open space.

By use of this approach, maximun of two injections were required to pack the aneurysms.

When GEL was injected into the aneurysm, it initially formed a thread-like structure and finally formed a large round structure which filled the entire lumen of the aneurysm. Both the balloon catheter and the microcatheter could be easily removed after injection of the GEL, which enabled further packing of the aneurysm by re-advancing the microcatheter and re-injecting the mixture with re-inflation of the balloon catheter **(Fig. 6).**

The lumen in a microcatheter could be cleaned by flushing with Lipiodol. However, when a guidewire was used to re-insert the microcatheter, GEL was occasionally found to remain in the hub portion of a microcatheter, which made insertion of the guidewire difficult. In this situation, another microcatheter was required.

Post-packing angiography revealed near complete packing of the aneurysm in 15 out of 18 aneurysms **(Fig. 4)**. No migration or leakage of GEL was observed for 30 min after packing.

Among different ratios of G.E.L. the most effective for aneurysms occlusion seemed to be 2:0,5:1 which offers the best

compromise between polymerization time, balloon resistance and catheter patency.

Post procedure angipgraphic controlos showed a stable occlusion of all the aneurysm with no signs of recurrence **(Fig. 5)**. The controls have been performe at at 7-, 30-, and 90-days after the embolization, according with the protocol.

Macroscopic study

The different subjects did not exhibit symptoms related to a painful state. During the surgery for removing the experimentally induced defects, no alterations due to an ongoing inflammatory process were evidenced. **(Fig .7)**

The diameter of the experimentally induced aneurysms ranged from 5 cm to 30 cm (mean diameter 12.4 cm ± 6.2 cm). A complete occlusion was observed starting from time 0 **(Fig 8A**) and remained evident at 7 **(Fig 8B**), 30 (**Fig 8C**), and 90 (**Fig 8D**) days post-surgery. The aneurysm occlusion was due to a mixture of the embolic agent and thrombotic materials.

Changes in the embolic cast

The distribution of the embolic material inside the embolic cast was homogeneous and the embolizing material adhered to the aneurysm wall. There was no evidence of recanalization of the embolic cast in samples examined, particularly after 7-, 30-, and 90-days post-surgery.

Vascular changes

Vascular integrity of the aneurysm wall was observed in all experimentally induced defects and no evidence of perivascular extravasation of embolic material was detected in any specimens.

An acute inflammatory reaction characterized by presence of hemorrhages was detected in all samples resected after few hours (t0) and 7 days post-surgery **(Fig 9A).** In samples collected after 7 days post-surgery, angionecrosis demonstrated by alteration of elastic fibers was also observed **(Fig, 9B).**

At 30 days post-surgery, the aneurysm wall was slightly thickened due to the proliferation of a poorly differentiated connective tissue and presence of a chronic vascular inflammation **(Fig 9C).** In the aneurysm wall scattered foreign body reactions at the periphery of embolizing material residues and characterized by the presence of foreign body giant cells were also detected **(Fig 9D)**. At the periphery of the aneurysm wall there was edema and host tissue showed a reduced inflammatory infiltration **(Fig 9E).**

After 90 days post-surgery the aneurysm wall was thickened and consisted of a well-differentiated connective tissue **(Fig**

9F). Pigment deposits of hemoglobin origin (both hemosiderin and hematin) were also observed.

Perivascular changes

In samples collected 7 days post-surgery a limited perivascular oedema was observed. After 30 days post-surgery a marked reduction of the perivascular oedema was evident, scattered perivascular inflammatory infiltrates constituted by macrophages and small lymphocytes were also present. No perivascular changes were detected in samples collected 90 days post-surgery.

Immunohistochemical studies

Immunohistochemical studies revealed the presence of a reduced number of T- and B- lymphocytes in the inflammatory infiltrates observed in the aneurysm wall after 7- and 30-days post-surgery **(Fig 10A)**. Many macrophages were detected in chronic vascular inflammatory infiltrates detected in the aneurysm wall after 30 days post-surgery **(Fig 10B)**. The number of macrophages in the aneurysm wall was drastically reduced in samples collected 90 days pot-surgery.

Morphometric analysis

Morphometric analysis performed to determine the thickness of the aneurysm wall at different time from embolization revealed that at 7 days post embolization, the aneurysm walls were only slightly increased due to the acute inflammatory reaction to the mixture deposit without significant differences (65μm ± 9 μm at t0 vs 120 μm ± 10 μm at t7). After 30 days post embolization there was a significant increase in the aneurysm wall thickening (120 μm ± 10 μm al t7 vs 366 μm ± 83 μm at t30; $p<0.001$) due to the connective tissue proliferation more evident 90 days after embolization (366 μm ± 83 μm al t30 vs 701 μm ± 261 μm at t90; $p<0.001$). **(Fig. 11)**

Discussion

Endovascular treatment of cerebral aneurysms has become an alternative to conventional neurosurgical clipping. The advent of Guglielmi Detachable Coils (GDC) provided safe and effective treatment for a large population of cerebral aneurysms [16]. Some anatomical and physical limitations and difficulties in dense packing associated with the use of GDCs in aneurysms have been identified because GDCs are less effective in wide-necked or large or giant aneurysms [17]. In this type of aneurysm, it is difficult to achieve complete packing and control of the aneurysm inflow with GDC technology.

Fernandez et al. [18] reported that complete aneurysm embolization using micro coil embolization could be achieved for 85 % of aneurysms with a neck smaller than 4 mm, but for only 15 % of aneurysms with a neck ≥4 mm. Debrun et al. [19] reported that 77 % of aneurysms with a dome-to-neck ratio of ≥2.0 could be occluded by use of micro coils compared with 53 % of aneurysms with a dome-to-neck ratio of <2.0.

Use of liquid embolic agents is a possible alternative for the treatment of some cerebral aneurysms [20-21]. However, these therapeutic techniques have not been generally accepted owing to intrinsic technical limitations. One major limitation has existed in the delivery mode of liquid embolic agent into the sac of the aneurysm without any possibility of repositioning or retrieval [22].

Some experimental and clinical methods have been tested to decrease distal migration of the liquid embolic agent, such as intra-aneurysmal flow control with proximal balloon protection, balloon inflation across the neck of the aneurysm, and use of metallic stents [23-24]. Kawai et al. described successful packing of a narrow-neck carotid artery aneurysm with NLE (n-butyl 2-cyanoacrylate + Lipiodol + Ethanol), under blood flow control with balloon inflation at the proximal end of the carotid artery. However, their method could not be used for wide-neck aneurysms because of the risk of NLE migration during packing.

Furthermore, in clinical use of balloon-assisted packing of an aneurysm, the balloon was inflated at the aneurysm site, to avoid migration, but not at the proximal end of the parent artery.

A step forward has been done with the study of Tanaka et al, in which NLE packing of aneurysms with a neck width of ≥6.3 mm and a dome-to-neck ratio of <2.0 was successfully performed with balloon inflation at the aneurysm site.

Balloon-assisted injection of liquid embolic agents, as well as both balloon or stent assisted coiling (BAC and SAC), uses a two-catheter system with balloon assistance; however, glue/dimethyl sulfoxide–compatible catheters and balloons are required, and the aneurysm sac is embolized with a liquid agent (Onyx or Glue) instead of a metallic coil.

The CAMEO trial, which investigated the treatment of 100 aneurysms with the Onyx liquid embolic system, found that occlusion rates were comparable to those seen for BAC and SAC, with 79% complete occlusion, 13% subtotal occlusion, and 8% incomplete occlusion at 12 months. However, the rate of serious adverse events was significantly higher at 26.8% [25]. Due to the high complication rate, this technique failed to gain traction in the United States, but it remains in practice in other countries.

As we know Glubran + Lipiodol mixture is not appropriate for packing of wide-neck aneurysms because it forms small oily droplets

and adheres firmly to the microcatheter and balloon catheter [26-27-28], which could prevent its removal from the vessel. In contrast, GEL seemed an appropriate material for aneurysm packing because it formed a single large solid droplet [29]. Compared with G+L, GEL was less adherent to the microcatheter and balloon catheter, which enabled re-advancement of the microcatheter into the aneurysm and re-injection of GEL, for additional packing via the microcatheter. The minimum adhesion of the GEL enabled easy removal of both catheters. We observed resistance of the microcatheter during retrieval in two of 18 aneurysms, although there was no sticking of the microcatheter to the glue cast.

The GEL mixture in our study had also excellent visibility under fluoroscopy during embolization. In addition to the optimal visibility of liquid embolic agents, these agents should be used under high-quality, subtracted, real-time fluoroscopic guidance to avoid leakage into the parent artery and distal migration of the glue.

During the aneurysm packing, continuous injection of GEL was necessary to prevent blood regurgitation. Ethanol is believed to accelerate polymerization along the GEL mixture–blood interface where NBCA molecules could bind to anions present in the blood [30].

Rapid polymerization along the surface of the NBCA mixture might help to reduce unwanted adhesion of the NBCA to the catheter.

Direct extension of our results to clinical study may have limitations because the hemodynamics of the surgically constructed side-wall aneurysm model in swine are different from those of a human aneurysm. In addition, the safety and stability of the embolic effect need to be confirmed after very long-term follow-up.

Taking everything to account, glue embolization of aneurysms is technically feasible with neck protection of the flow with balloon. Although among different ratios of G.E.L. the most effective for aneurysms occlusion seemed to be 2:0,5:1 which offers the best compromise between polymerization time, balloon resistance and catheter patency.

Conclusion

This is the first experimental study to address feasibility, efficacy and tissue reaction and presence of occlusion after embolization with a mixture of n-butyl-cyanoacrylate (Glubran 2®), Ethanol and Lipidol® of aneurysms experimentally induced in swine.

At seven days after the embolization, a mild acute vascular reaction was evident in the aneurysm wall, probably related with the polymerization of cyanoacrylic glue present in the mixture, without involvement of host tissues at the periphery of experimentally induced lesion.

This acute reaction was followed by a chronic inflammation which induced a thickening of aneurysm wall due to proliferation of connective tissue still confined within the aneurysm wall.

The embolization procedure induced vascular changes followed by a complete and stable occlusion of the aneurysms. Our experimental study demonstrated that the presence of the occlusion remained stable up to 90 days after surgery, without recanalization phenomena.

Regarding possible future clinical applications, although requiring balloon occlusion and selective catheterization, GEL may be valuable for embolization of arteriovenous malformations and control of acute bleeding and for isolation of aneurysms. GEL could also shorten the treatment time according to its very short polymerization time and reducing the overall costs of the procedure. It is possible that GEL could be used in combination with detachable coils for aneurysm packing.

In conclusion, although limitations exist, as the ratio of ethanol affect GEL configuration to a single large-sized droplet, packing for aneurysm using GEL is feasible and safe as it becomes more solid with higher occlusive ability compared with simple Glubran and lipiodol mixture, with negligible adhesion to the microcatheter and embolic complication. Among different ratios of G.E.L. the most effective for aneurysms occlusion seemed to be 2:0,5:1 which offers the best compromise between polymerization time, balloon resistance and catheter patency.

Conflict of Interest Statement

The author declares no conflicts of interest.

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Figure Legends

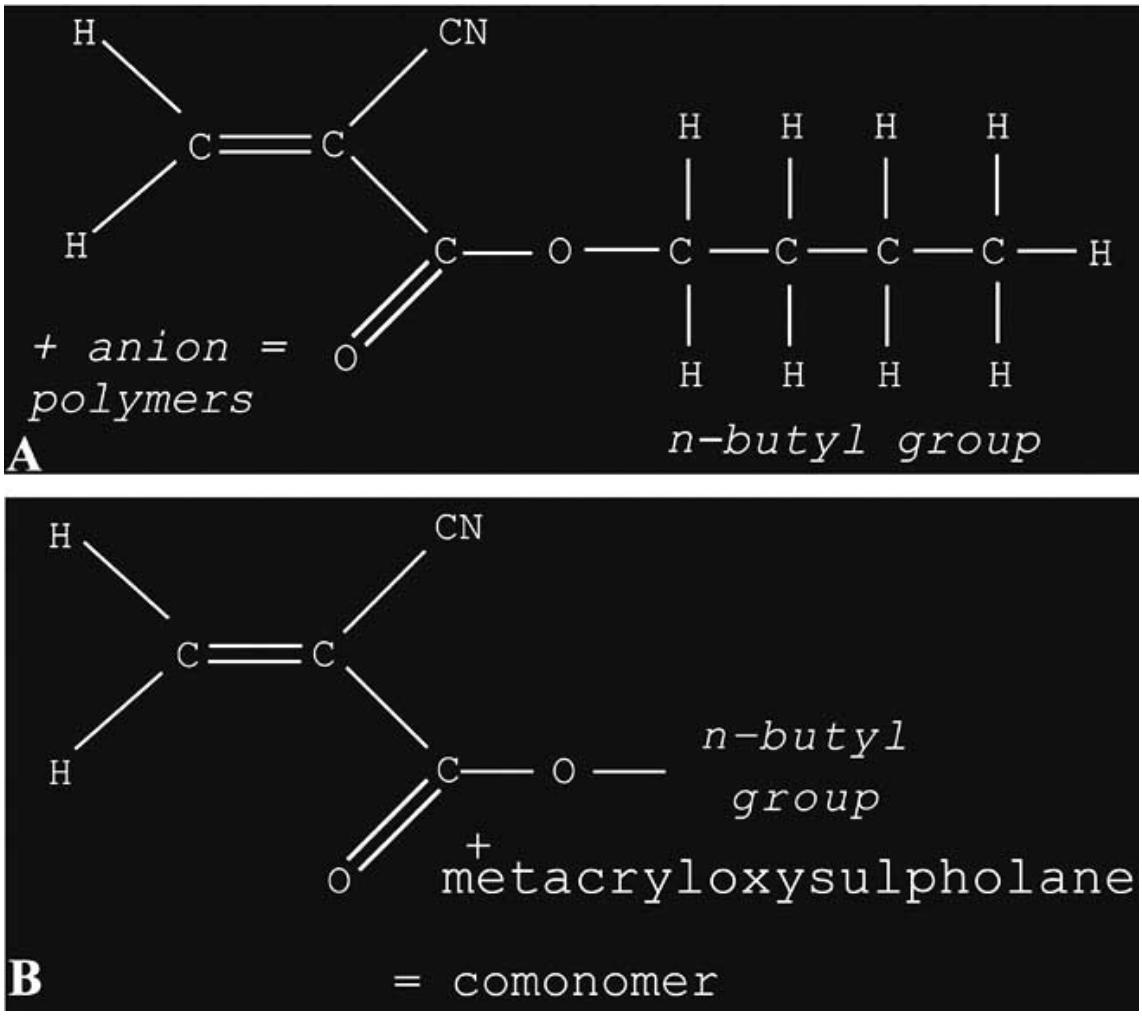

Figure 1. Chemical structure of two acrylic glues (n-butyl cyanoacrylates).

A. Histoacryl®: N-butyl 2-cyanoacrylate (NBCA).

B. Glubran 2®: N-butyl 2-cyanoacrylate (NBCA) + metacrylosulpholane (NBCA-MS).

Figure 2. Hybrid Operating Room used for both surgery and embolization

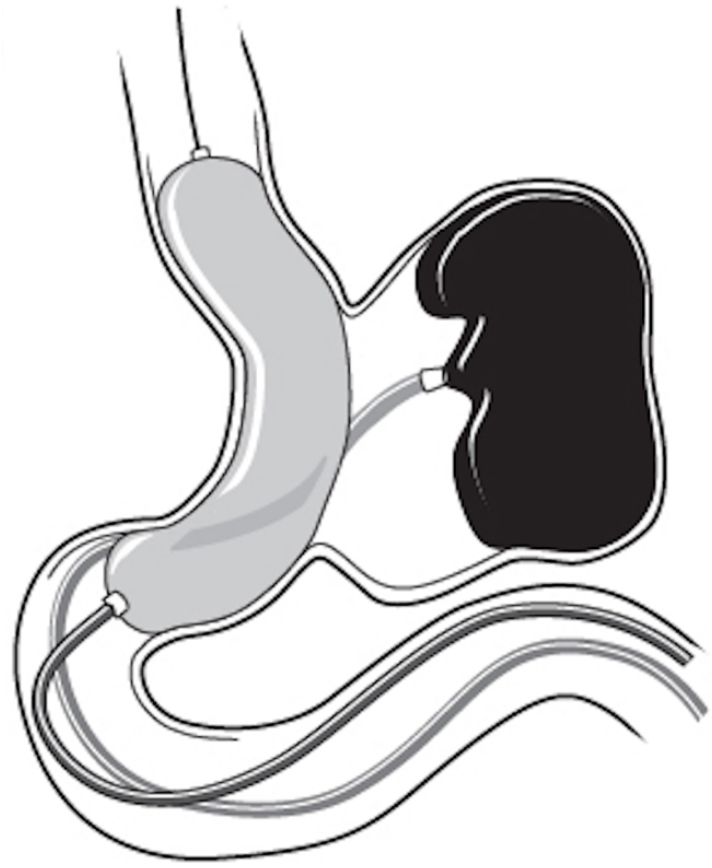

Figure 3. Ballon-assisted liquid embolic embolization

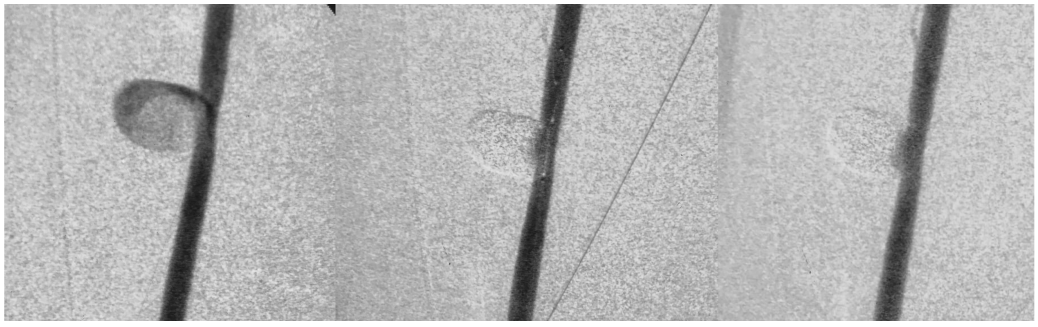

Figure 4. Carotid angiography shows the aneurysm before embolization **(a)** and attempted embolization using GEL 2:0,5:1, via a microcatheter inserted into the microaneurysm **(c)** with balloon protection **(b).**

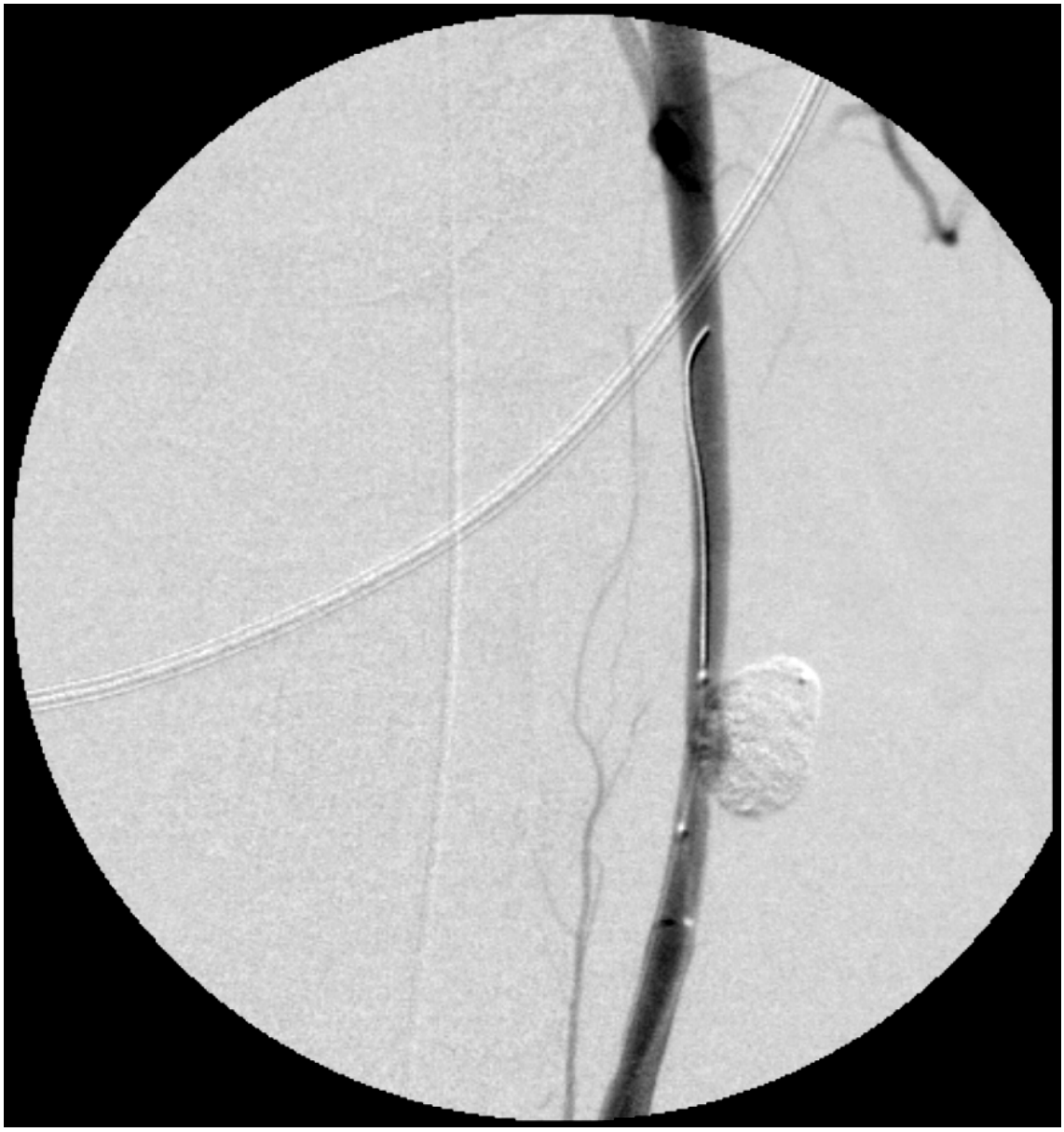

Figure 5: An angiographic intraoperative control after embolization with balloon deflated

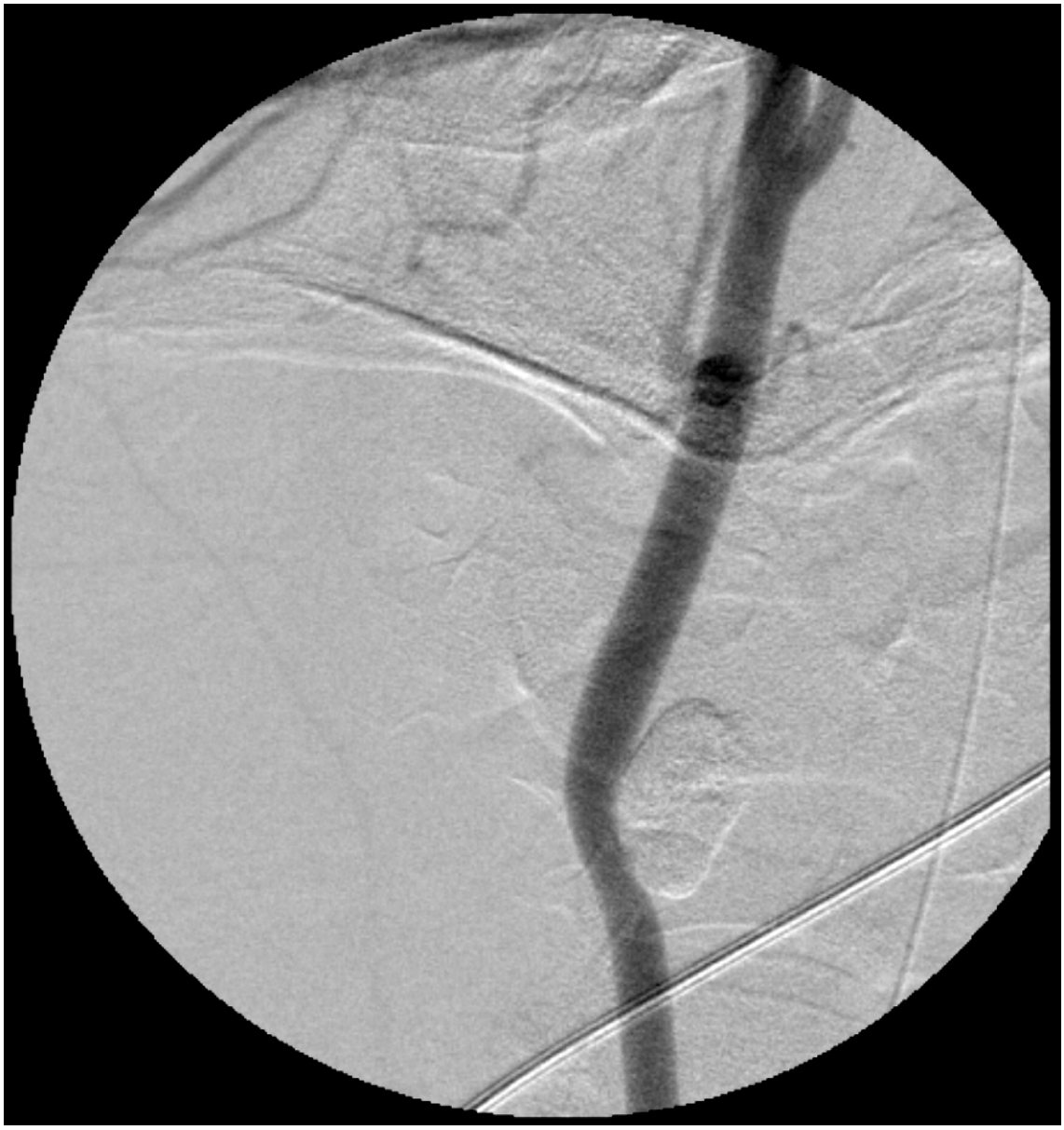

Figure 6. Follow-up control at 90 days. Occlusion remains stable

Figure 7. Aneurysms excised

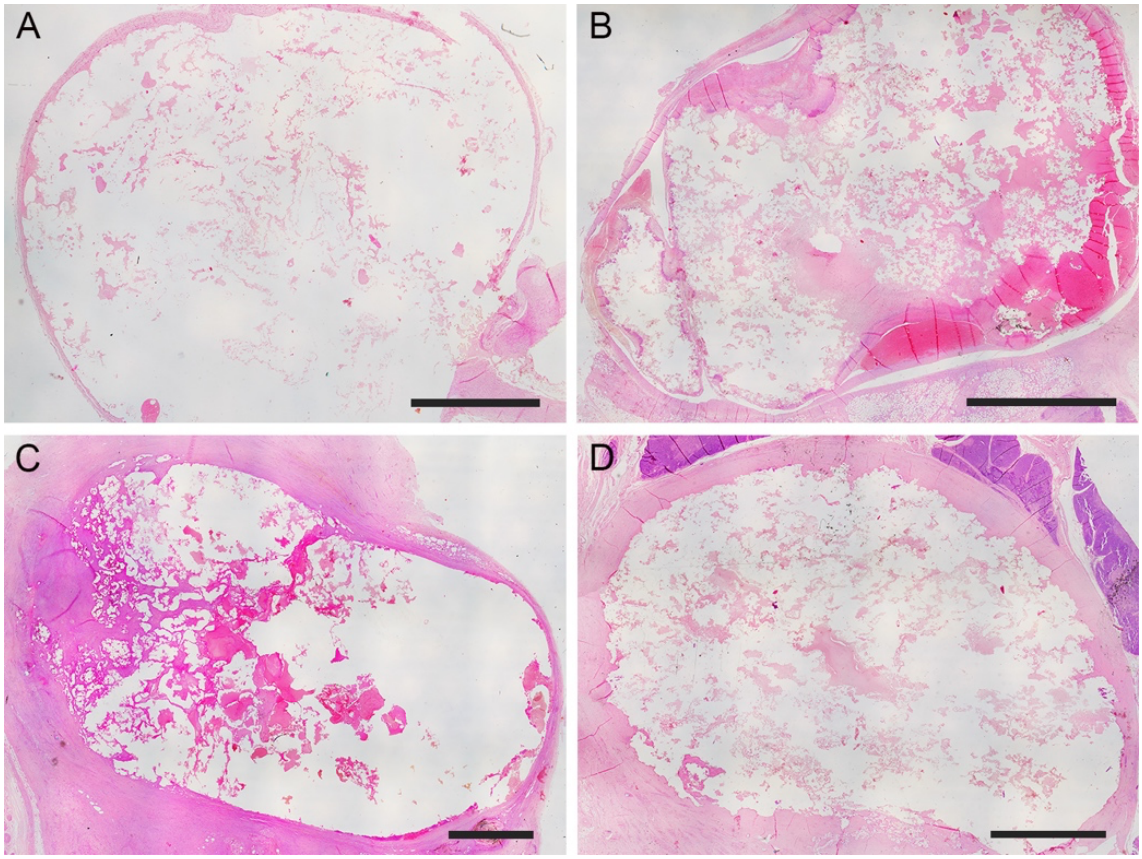

Figure 8. Aneurysms embolized using the modified mixture. A) After the embolization a complete occlusion due to the embolizing mixture resulting as a refractile material was evident (bar = 0.5 cm). B) After 1 week after embolization the aneurysm lumen was filled by the embolizing mixture mixed with fibrin, small hemorrhagic areas are evident close to the wall of the structure (bar = 1 cm). C) After 30 days from surgery the aneurysm was completely occluded by the modified mixture (bar =0.5 cm). D) After 90 days after emolization the aneurysm lumen was still occluded, and recanalization phenomena are not evident (bar = 0.5 cm).

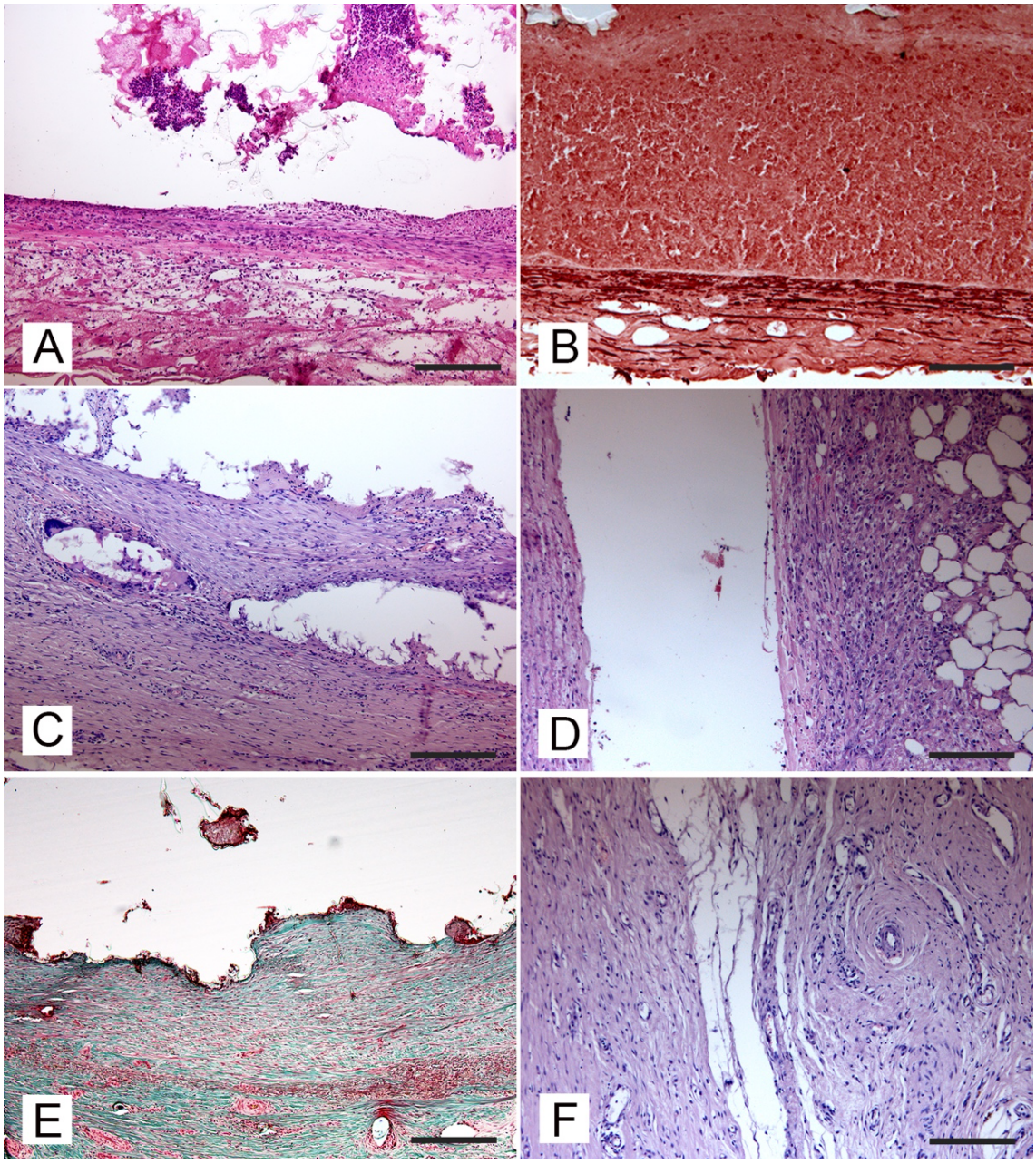

Figure 9. Aneurysms wall at different time after embolization, A and B after one week, C and D after 30, and E and F after 90 days: A) Aneurysm wall after one week after emobilization, acute inflammatory reaction with widespread hemorrhages and a diffuse presence of neutrophils (H-H, bar = 50 micron); B) Aneurysm wall after one week after emobilization , alteration of elastic fibers in the aneurysms wall (Orcein stain, bar = 100 micron); C) Aneurysm wall 30 days after emobilization, thickening of the aneurysm

wall due to immature connective tissue, presence of small glue deposits delimited by a chronic inflammatory reaction (H-H, bar = 100 micron); D) Aneurysm wall 30 days after emobilization, host tissues at the periphery of experimental aneurysm showed a reduced inflammatory reaction (H-H, bar = 200 micron); E) Aneurysm wall 90 days after emobilization, the aneurysm wall was constituted by well differentiated connective tissue with a reduced inflammatory reaction (Masson trichrome Goldner stain, bar = 100 micron); F) Aneurysm wall 90 days after emobilization, absence of edema and inflammatory phenomena at the periophery of the aneurysm wall (H-H, bar = 200 micron).

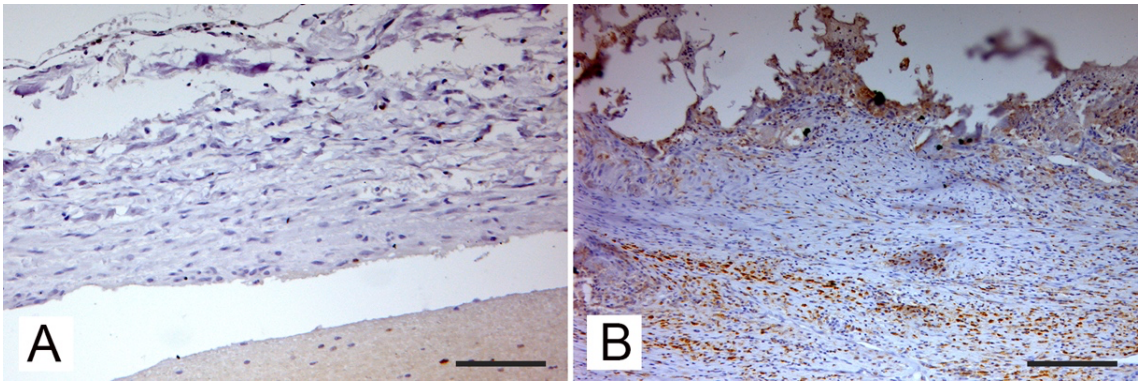

Figure 10. Aneurysm wall at different time after embolization, immunohistochemical localization of T lymphocytes and macrophages. A) Immunohistochemistry for CD3 positive T lymphocytes. Scattered T lymphocytes are detectable in the aneurysm wall at one week after embolization (IHC using an anti CD3 primary antibody, hematoxylin counterstain, bar = 200 micron); B) Immunohistochemistry for Iba1 positive macrophages. Several Iba1 positive macrophages within the aneurysm wall after 30 days after embolization (IHC using an anti Iba1 primary antibody, hematoxylin counterstain, bar = 100 micron)

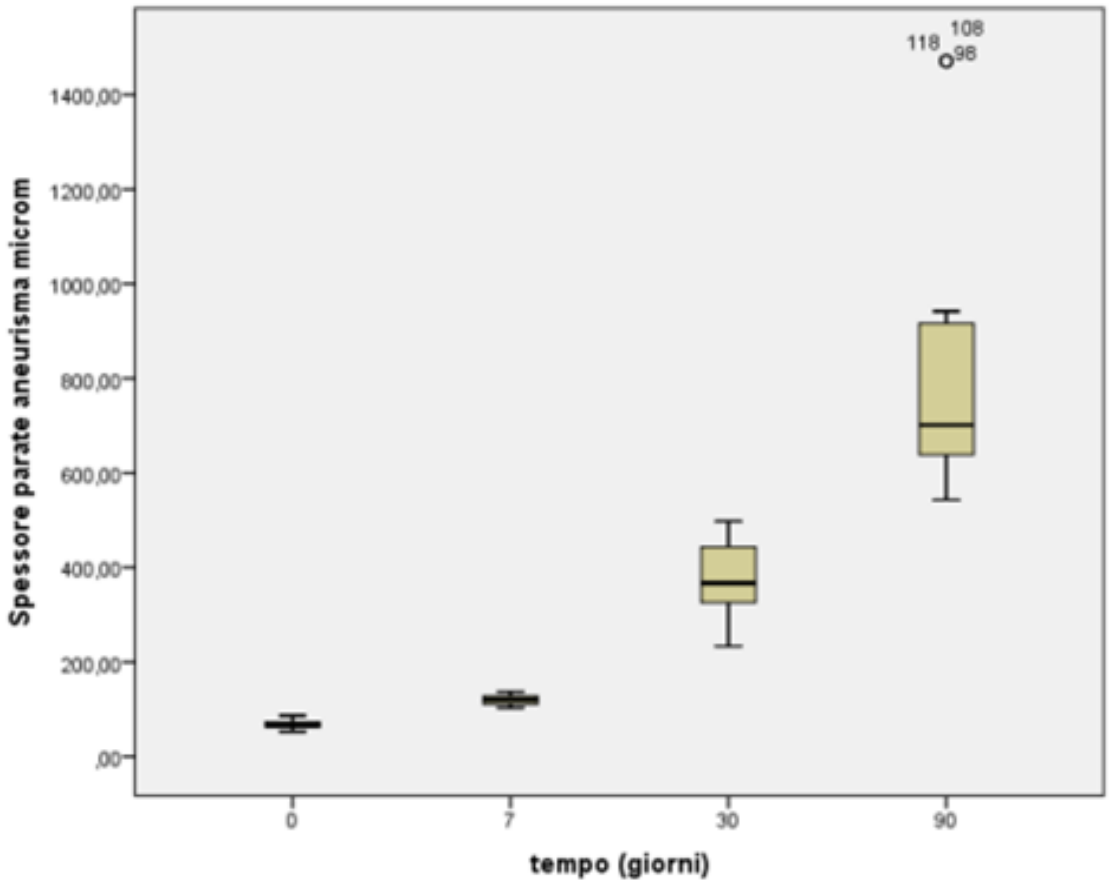

Figure 11. Vessel wall thickening analysis

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Ai miei genitori