Intracisternal BioGlue injection in the fetal lamb: a novel model for creation of obstructive congenital hydrocephalus without additional chemically induced neuroinflammation

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OBJECTIVE The authors hypothesized that new agents such as BioGlue would be as efficacious as kaolin in the induction of hydrocephalus in fetal sheep.

METHODS This study was performed in 34 fetal lambs randomly divided into 2 studies. In the first study, fetuses received kaolin, BioGlue (2.0 mL), or Onyx injected into the cisterna magna, or no injection (control group) between E85 and E90. In the second study, fetuses received 2.0-mL or 2.5-mL injections of BioGlue into the cisterna magna between E85 and E90. Fetuses were monitored using ultrasound to assess lateral ventricle size and progression of hydrocephalus. The fetuses were delivered (E120–E125) and euthanized for histological analysis. Selected brain sections were stained for ionized calcium binding adaptor 1 (Iba1) and glial fibrillary acidic protein (GFAP) to assess the presence and activation of microglia and astroglia, respectively. Statistical comparisons were performed with Student’s t-test for 2 determinations and ANOVA 1-way and 2-way repeated measures for multiple determinations.

RESULTS At 30 days after injection, the lateral ventricles were larger in all 3 groups that had undergone injection than in controls (mean diameter in controls 3.76 ± 0.05 mm, n = 5). However, dilatation was greater in the fetuses injected with 2 mL of BioGlue (11.34 ± 4.76 mm, n = 11) than in those injected with kaolin (6.4 ± 0.96 mm, n = 7) or Onyx (5.7 ± 0.31 mm, n = 6) (ANOVA, *p ≤ 0.0001). Fetuses injected with 2.0 mL or 2.5 mL of BioGlue showed the same ventricle dilatation but it appeared earlier (at 10 days postinjection) in those injected with 2.5 mL. The critical threshold of ventricle dilatation was 0.1 for all the groups, and only the BioGlue 2.0 mL and BioGlue 2.5 mL groups exceeded this critical value (at 30 days and 18 days after injection, respectively) (ANOVA, *p ≤ 0.0001). Moderate to severe hydrocephalus with corpus callosum disruption was observed in all experimental groups. All experimental groups showed ventriculomegaly with significant microgliosis and astrogliosis in the subventricular zone around the lateral ventricles. Only kaolin resulted in significant microgliosis in the fourth ventricle area (ANOVA, *p ≤ 0.005).

CONCLUSIONS The results of these studies demonstrate that BioGlue is more effective than Onyx or kaolin for inducing hydrocephalus in the fetal lamb and results in a volume-related response by obstructive space-occupancy without local neuroinflammatory reaction. This novel use of BioGlue generates a model with potential for new insights into hydrocephalus pathology and the development of therapeutics in obstructive hydrocephalus. In addition, this model allows for the study of acute and chronic obstructive hydrocephalus by using different BioGlue volumes for intracisternal injection.

https://thejns.org/doi/abs/10.3171/2019.6.PEDS19141

KEYWORDS congenital hydrocephalus; experimental model; fetal lamb; BioGlue; kaolin; Onyx; ventriculomegaly; neuroinflammation
Congenital hydrocephalus is one of the most common congenital anomalies, with an incidence ranging from 0.3 to 0.8 per 1000 births. It is also the most common reason for brain surgery in children. Hydrocephalus results from increased intracranial pressure and dilation of the ventricles and, left untreated, most children with congenital hydrocephalus will most likely die. With standard CSF shunting or neuroendoscopic treatment, most children with hydrocephalus will survive. However, the outcome of congenital hydrocephalus can be poor and challenging, including surgical complications as well as cognitive problems, poor academic achievement, and neurological sequelae, which are the most important problems among long-term survivors.

Hydrocephalus, as a multifactorial disease, has multiple competing classifications, which makes it difficult to study. When hydrocephalus occurs secondary to another condition, such as hemorrhage, infection, or neoplasm, it is usually called acquired (extrinsic) or secondary hydrocephalus. When hydrocephalus occurs as a developmental process (intrinsic hydrocephalus), different obstructive points can be distinguished. Fetal or congenital hydrocephalus research can contribute to the understanding of the pathophysiology of hydrocephalus and help to identify potential pharmacological and/or therapeutic interventions that may improve outcomes in the future.

Experimental studies to induce hydrocephalus in animals were started a century ago and are still conducted today. Many types of animals have been used, including mice, rats, cats, dogs, sheep, and monkeys. Some recent studies that may improve outcomes in the future.

Methods

Animal Preparation

Pregnant sheep around day 80 of gestation were transferred to the animal facility 1 week prior to the study for acclimatization and health screening. All animals received an intramuscular injection of Tribrissen 48% (trimethoprim and sulfadiazine) 1 day prior to surgery and methoprim and sulfadiazine 1 day prior to surgery. Sedation was induced with intravenous propofol injected via the cephalic or jugular vein (5 mL/kg). The animals were anesthetized via tracheal tube with 2%–3% isoflurane in O2 using a respirator. About 30 minutes prior to surgery, the animals were administered an intravenous infusion of 1 g of cefazolin in 100 mL of saline. In addition, each animal received an intramuscular injection of Temgesic (buprenorphine 0.005 mg/kg) and a Duragesic (fentanyl) patch (50 mg) preoperatively; the patch was replaced 3 days after surgery if required.

Access to Fetal Cisterna Magna via Open Uterine Surgery

The surgery was performed under sterile conditions at 85–90 days of gestational age. The maternal laparotomy site was shaved and prepared with 70% alcohol and a povidone-iodine scrub. A laparotomy was performed, and the uterus was exteriorized and fixed with amniotic membrane anchoring before exteriorization of the fetal head. After manual head fixation and under direct palpation, a 22-gauge needle was introduced into the posterior neck, caudal to the exterior occipital protuberance. An previously described by Edwards et al. and Johnston et al., the needle with catheter was inserted into the subarachnoid space and 1–2 mL of CSF (the same volume as the volume to be injected) was withdrawn (Fig. 1A–C); then, the sterile reagent was injected manually, slowly (over the course of 1 minute) into the cisterna magna (Supplemental Fig. 1). After the injection and appropriate hemostasis, the fetal lamb was re-housed in utero, and warm Ringer’s lactate solution with cephalosporin was used to refill the uterine cavity. The uterus was closed with a running 2-0 Vicryl suture, and the maternal abdominal wall was closed in layers. Postsurgical monitoring was maintained until the ewe was fully recovered.

Animal Groups and Injected Agents

The agents used were kaolin (aluminum silicate...
(Al$_2$Si$_2$O$_5$[OH]$_4$) kaolin powder, Sigma); Onyx (Medtronic/ev3), a polyvinyl-alcohol product used for endovascular embolization therapy; and BioGlue (CryoLife Inc.), which is used as a hemostatic and adhesive agent.

BioGlue has an exclusive spiral dispenser to mix its components before delivery of the product. We connected this special tube to the needle by cutting the tip of this applicator (Fig. 1A, image e).

**Study 1**

In this study, we compared the use of different substances to induce hydrocephalus in 24 fetal lambs assigned randomly to 4 groups—3 experimental groups and 1 control group. Lambs in the kaolin group (n = 7) underwent percutaneous injection of 1 mL of 2% (20 mg/mL) kaolin (aluminum silicate [Al$_2$Si$_2$O$_5$[OH]$_4$], kaolin powder) into the cisterna magna (Fig. 1A, image d). Lambs in the Onyx group (n = 6) underwent percutaneous injection of 2 mL of Onyx solution into the fetal cisterna magna (Fig. 1A, image f). Lambs in the BioGlue group (n = 6) underwent percutaneous injection of 2.0 mL of BioGlue into the cisterna magna (Fig. 1A, image e). The control group was made up of normal fetal lambs (n = 5), co-twins of lambs injected with one of the experimental agents. Thus all lambs in the control group were exposed to the same anesthesia protocol and uterine surgery but did not undergo any injection into the cisterna magna.

**Study 2**

This study investigated the dose-dependent response based on hydrocephalus development in 10 fetal lambs that were divided randomly into 2 groups as follows: group 1 (BioGlue 2.0 mL), percutaneous injection of 2.0 mL of BioGlue into the fetal cisterna magna (n = 5); and group 2 (BioGlue 2.5 mL), percutaneous injection of 2.5 mL of BioGlue into the fetal cisterna magna (n = 5).

**Ultrasound Monitoring**

All fetuses were monitored with ultrasonography (by means of a transabdominal convex transducer [C5-2 at 2–5 MHz, Philips ATL HDI 5000]) before and after injections into the cisterna magna. Following surgery, weekly ultrasound examinations were performed to monitor hydrocephalic progression and any complications, the fetal cardiac rate, and brain/ventricular remodeling, as well as to perform related measurements, until the animals were euthanized at 120–125 days of gestation.

Measurements were performed in the true axial plane in the atria of the lateral ventricle and glomus of the choroid plexus.

Fetal/neonatal measurements included a) lateral ventricle diameter (LVD), measured from the inner margin of the medial ventricular wall to the inner margin of the lateral wall; b) biparietal diameter (BPD); c) head circumference...
and d) ratio of LVD to BPD. We developed a scale, the Cincinnati Hydrocephalus Severity Scale (CHSS), for assessment of hydrocephalus in neonatal lambs, with categories ranging from normal to severe, as determined by LVD and LVD/BPD ratio. The measurement ranges for each severity category are shown in Figs. 2 and 3.

**C-Section and Harvest of the Neonatal Lamb Brains**

Once significant ventriculomegaly was achieved, approximately 4–6 weeks following injection (i.e., at around 120–125 days of gestation), C-section was performed. This procedure was done under maternal anesthesia as described above.

The neonatal lambs were euthanized with 20 mL of 240-mg/mL pentobarbital, administered intravenously, and their brains were harvested and cut along the midline into 2 halves for histological analysis.

**Magnetic Resonance Imaging**

MRI was performed on the pregnant ewes and newborns to look for hydrocephalic changes in the fetal lambs. Image acquisition was carried out using a 1.5-T MR scanner with a Sense body 4-channel coil (Intera, Philips Medical Systems) for pregnant ewes and Sense Flex small coil (Intera, Philips Medical Systems) for newborn lambs. The sequences were acquired with high-definition T1/SE (repetition time [TR 450–650 msec]/echo time [TE 15 msec]/echo train length/acquisition) and T2/TSE (repetition time [TR 3778 msec]/echo time [TE 110 msec]/echo train length/acquisition) with respiratory compensation, 2-mm thickness slices, and a 384 × 240–mm acquisition matrix.

**Morphological and Histological Analysis**

One half of each brain was submerged in formalin for 48 hours and embedded in a paraffin block after 70% alcohol washes.

Histological techniques were performed on 5-μm-thick sections that included the periventricular region, brainstem, and cerebellum. Hematoxylin and eosin staining was performed using a Varistain Gemini ES autostainer (Thermo Scientific). A 1:4 mixture of Mayer's hematoxylin (Lillie's modification) histological staining reagent (Dako, no. S3309) and Automation hematoxylin staining reagent (Dako, no. S3301) was used with 0.25% Eosin-Y (Richard Allen Scientific, no. 71225) for contrast.

**Immunofluorescence**

Immunofluorescence staining was performed on brain sagittal paraffin sections. The sections were dried, followed by permeabilization with 0.1% Triton X-100 (Sigma Aldrich) in phosphate-buffered saline (PBS), blocked for nonspecific binding for 1 hour with 5% bovine serum albumin (BSA) in PBS, and then incubated with primary antibody anti-GFAP (glial fibrillary acidic protein) (Abcam, no. AB4674) (1:500) and anti-Iba1 (ionized calcium binding adaptor 1) (Wako, no. 19-19741) (1:1000) overnight at 4°C in a humid chamber. Sections were washed and incubated for 1 hour with Alexa Fluor 488–, Alexa Fluor 568–, or Alexa Fluor 647–conjugated secondary
antibodies (Life Technologies) (1:1000) in a humid chamber at room temperature. Slides were washed, mounted with DAPI mounting media (SouthernBiotech, no. 0100-20), and visualized with a Nikon fluorescent microscope (Nikon Inc).

Immunolabeled Cell Counts and Area Measurement

Iba1+ and GFAP+ cell counts and immunostained area measurement were performed using NIS Elements AR 4.5 software (Nikon Instruments Inc.). For each animal, mean counts and area measurements were obtained in 10 randomly selected frames from specific regions of interest in 3 consecutive slides for each of the following structures: the peri-lateral ventricular area, the brainstem, and the cerebellum. Percentages of immunopositive cells were calculated as the total number of cells in a selected area divided by the number of immunopositive cells in that area (area measured in pixels squared [px^2]). All quantifications were performed by an investigator blinded to the experimental groups.

Statistical Analysis

Data are expressed as mean and standard deviation, and a p value < 0.05 was considered statistically significant. Intrigroup and intergroup comparisons of the same variable for multiple determinations were performed with ANOVA 1-way and 2-way repeated measures, followed by pairwise Tukey’s multiple comparisons test. The GraphPad Prism 8 package was used for graph and statistical calculations.

Results

Comparable Rates of Fetal Demise Were Seen in All Experimental Groups

The rapid polymerization of BioGlue and Onyx forms solid masses that can potentially damage the brainstem of the fetus by acute compression. For this reason and to avoid increasing pressure by adding fluid volume, before each injection into the cisterna magna we withdrew a volume of CSF equivalent to the volume injected. Despite this precaution, we observed deaths of fetuses in all experimental groups (similar rates) and none in the control group. Details are provided in Table 1.

All Injections Resulted in Severe Hydrocephalus and Corpus Callosum Disruption by MRI Assessment

Kaolin, Onyx, and BioGlue injections all were followed by development of hydrocephalus in fetal lambs...
Oria et al. (Fig. 1B, image a). Axial and sagittal cerebral MR images obtained after delivery showed severe hydrocephalus and corpus callosum disruption (Fig. 1B, image c) in the lambs that had been subjected to fetal injections compared with normal brain development in untreated, control newborn lambs (Fig. 1B, image b).

**Pathology**

Coronal dissection of the cranium after delivery showed moderate to severe hydrocephalus and corpus callosum disruption in all injection groups (Fig. 1B, image d).

**Ventricular Dilatation Was Observed in Hydrocephalic Fetuses in All Injection Groups**

We observed lateral ventricular dilatation by ultrasound in all experimental groups in study 1, most significantly following BioGlue injection (Table 1). All injection groups demonstrated a significant increase in LVD 30 days after injection compared with controls (5.8 ± 0.72 mm for kaolin, 5.4 ± 0.73 mm for Onyx, and 11.34 ± 4.53 mm for BioGlue 2.0 mL vs 3.8 ± 0.08 mm for the control group; ANOVA, p ≤ 0.0001). The mean LVD in the BioGlue group at 30 days after injection was greater than that in the Onyx or kaolin group (p ≤ 0.001). A BioGlue injection of 2.0 mL induced a significant (ANOVA, p ≤ 0.0001) increase in LVD (mean LVD 7.36 ± 2.44 mm, moderate hydrocephalus) even at 23 days after injection (vs mean 3.54 ± 0.14 mm in controls); a significant increase was not seen this early in the other treatment groups (Fig. 2A, image a).

No statistically significant between-group differences were found for HC or BPD. Moreover, all experimental groups followed the same developmental growth trajectory as the control group (Fig. 2A, images b and c).

**Severe Hydrocephalus Induction With BioGlue Injection**

Both groups injected with BioGlue (2.0 mL and 2.5 mL) demonstrated similar ventricle dilatation (mean LVD 11.34 ± 4.53 mm for the BioGlue 2.0 mL group and 11.47 ± 2.32 mm for the BioGlue 2.5 mL group), but the increase in LVD appeared earlier in the BioGlue 2.5 mL group, just 10 days after injection (vs 3.24 ± 0.09 mm in the control group; ANOVA, p ≤ 0.001) (Fig. 2B, image a).

No statistically significant differences were found between either BioGlue group and the control group with respect to HC or BPD (Fig. 2B, images b and c).

We calculated the mechanical compression critical threshold using the ratio of LVD to BPD (Table 1). The critical threshold was 0.1 for all the groups, but the BioGlue 2.0 mL and BioGlue 2.5 mL groups exceeded this critical value at 30 days and 18 days after injection, respectively (ANOVA, p ≤ 0.0001) (Fig. 3).

**Hydrocephalus Induced Astrogliosis in All Experimental Groups**

All experimental groups developed ventriculomegaly, hydrocephalus, and significant related astrogliosis as demonstrated by staining for GFAP in the subventricular zone (SVZ) around the lateral ventricles. Additionally, we found reactive astrogliosis in the exposed layers of the periventricular area all around the dilated ventricles, with signifi-
cant increases in GFAP expression when compared with specimens from control animals (ANOVA, $p \leq 0.005$ and $p \leq 0.001$) (Fig. 4A and B). Only kaolin induced astrogliosis, with more astrocytes and more area staining positive for GFAP, in the fourth ventricle area, brainstem (Fig. 5A and B), and cerebellum (Fig. 6A and B) (ANOVA, $p \leq 0.005$ and $p \leq 0.001$).

### Kaolin but Not the Other Agents Induced Local Chemical Microgliosis

To assess neuroinflammation in the hydrocephalic fetuses, we analyzed morphological changes in microglial cells. We observed the presence of reactive microgliosis in the periventricular area in hydrocephalic animals (Fig. 4A and B); this neuroinflammatory response correlated with the severity and size of the ventricle dilatation. Animals that received BioGlue injections showed more microgliosis, as demonstrated by a greater increase in Iba1+ cells compared with controls, than animals injected with the other experimental agents (ANOVA, $p \leq 0.005$ and $p \leq 0.001$) (Fig. 4B).

In the cerebellum, at the level of the fourth ventricle, we did not observe changes in microglia as determined by Iba1 immunofluorescence when comparing expression between the induced-hydrocephalus groups after delivery (Fig. 6A and B). In contrast, however, we did find significant differences in the brainstem at the fourth ventricle area; in this region we found kaolin-induced microgliosis, with an increased number of Iba1+ cells (ANOVA, $p \leq 0.005$ and $p \leq 0.001$) (Fig. 5A and B).

Even though Onyx did not induce a chemical inflammatory reaction in the fourth ventricle area, in some of the animals in the Onyx group we observed massive focal microglial aggregations in some outer areas of the cerebellum (Fig. 7) in the region of the cisterna magna when compared with controls. We also observed neuronophagia directed to Purkinje neurons, which were surrounded by microglia/macrophages, in some areas of the cerebellum in the Onyx group (Fig. 7).

### Discussion

There are many experimental animal models that recapitulate congenital hydrocephalus,\(^1\,5\,7\,11\,12\,16\,17\,19\,21\,24\,25\,29\,30\) However, some of these models are associated with multiple congenital abnormalities such as encephalocele, meningocele, and cranium bifidum due to teratogenic effect of the corticosteroid,\(^20\) which makes the results complex to interpret. Models for obstructive hydrocephalus via induction of aqueduct stenosis using injection of different
Compounds can be challenging due to the high viscosity of the compound. However, BioGlue and Onyx are easily injected, like kaolin, into the cisterna magna, as we observed in our studies. We were able to induce hydrocephalus with all 3 substances—kaolin, Onyx, and BioGlue.

Previous studies using kaolin have demonstrated a local chemical meningitis-like neuroinflammatory response, adding a second variable into the study of ventriculomegaly: an inflammatory reaction in addition to the mechanical obstruction of CSF flow. BioGlue has received approval for use in many vascular, pulmonary, and soft tissue repairs, and it has been used in cardiothoracic surgery for 20 years worldwide. The advantage of BioGlue is that the bifunctional glutaraldehyde molecule covalently binds BSA molecules to each other as well as to lysine in proteins on the cell surface and in the extracellular matrix. This reaction is spontaneous, increasing tensile and shear strength, and also leads to polymerization in a liquid medium. The albumin provides an extensive flexible network of bonds and forms a watertight mechanical seal. In this study, we have demonstrated that BioGlue formed solid masses, causing hydrocephalus by obstruction of the cerebrospinal circulation at the aqueduct of Sylvius and fourth ventricle levels with no added local neuroinflammatory reaction. However, we observed increased numbers of Iba1+ cells and increased area stained with Iba1 in BioGlue animals in the periventricular region, probably due to the severity of the disease with these doses (severe lateral ventricle dilatation). Further investigations with lower doses will reveal the correlation with microgliosis and lower lateral ventricle dilatation.

Onyx, an ethylene–vinyl alcohol copolymer, solidifies into a spongy nonadhesive embolic agent that forms a nonpermeable flexible mass in the presence of an ionic environment such as CSF. In our study, Onyx induced

![Immunofluorescence staining of GFAP and Iba1 in the fourth ventricle region.](image-url)

**A:** Sagittal sections stained with Iba1 (green) and GFAP (red) and co-stained with DAPI (blue); microgliosis determined with Iba1 and astrogliosis determined with GFAP immunostaining in the fourth ventricle area. Specimens from lambs with kaolin-induced hydrocephalus showed microgliosis in the brainstem (d, arrow) and had more astrogliosis than those in the control (a), BioGlue (b), and Onyx (c) groups. Original magnification ×400. **B:** GFAP-stained area and Iba1-stained cell count. Astrogliosis and microgliosis determinations in brainstem of animals in the kaolin group. Data are presented as means and SDs (error bars). *p ≤ 0.005; **p ≤ 0.001 (ANOVA). Figure is available in color online only.
the same degree of moderate hydrocephalus as kaolin, with the same neuroinflammatory reaction in the SVZ of the lateral ventricles. However, while Onyx is nonadhesive and produces less local inflammation than kaolin,\(^1\) the authors of one study observed an intense foreign body giant cell-type reaction in response to Onyx in the injection area.\(^2\) In contrast, we observed local microglial reaction in specific areas of the cerebellum that were in contact with the Onyx as well as in the cisterna magna but not in the fourth ventricle, likely due to the speed of polymerization. We observed Purkinje neuronophagia in animals that received Onyx injection, a neuropathological phenomenon observed in neurodegenerative processes.\(^4,6\)

In a reported fibrin glue (Tisseel) model, injection into the cisterna magna of the rat induced brainstem compression.\(^2\) In contrast, we did not observe brainstem compression following injection of Onyx or 2.0 mL or 2.5 mL of BioGlue, but it may happen if increased volumes are used. Importantly in our model, CSF was withdrawn prior to the injections to avoid changes in intracranial pressure.

One limitation of our study is the different degree of ventriculomegaly in the different groups and animals. Since astro-microgliosis in the ventricular area is described as a consequence of the hydrocephaly by distension and alterations of the brain ependyma, SVZ, and white matter, it can be difficult to compare it in different degrees of severity according to our CHSS.

In our model with BioGlue, we created a physical obstruction in fetal lambs around 85 days of gestational age, which corresponds to 20–22 weeks in human gestation, to mimic the developmental (intrinsic) form of congenital hydrocephalus during the second trimester in humans.

Conclusions

Our novel model of fetal hydrocephalus utilizing BioGlue is more effective than Onyx or kaolin for inducing hydrocephalus in the fetal lamb and produces a response that depends on the volume injected, without localchemical neuroinflammatory reaction. The model offers the potential for new insights into the impact of hydrocephalus on fetal brain development and may aid in the identification of therapeutic targets for obstructive hydrocephalus without confounding neuroinflammation. In addition, this model offers the opportunity to study both acute and chronic obstructive hydrocephalus by using different volumes of BioGlue injected into the cisterna magna of the fetal lamb.

Acknowledgments

We appreciate Holly Poling for her help with sample preparation.

This work was supported by Prof. Jose L. Peiro internal CCHMC Pediatric Surgery Division funding and JUMISC, Caceres (Spain). Soner Duru was supported with a research scholarship.
References

16. Khan OH, Del Bigio MR: Experimental models of hydro-
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cerebrospinal pressure, hydrocepha-
lus and blood pressure in the cat follow-
19. Michejda M, Hodgen GD: In utero diagnosis and treatment of non-human primate fetal skeletal anomalies. I. Hydrocepha-
21. Nakayama DK, Harrison MR, Berger MS, Chinn DH, Halks-
Miller M, Edwards MS: Correction of congenital hydro-
cephalus in utero I. The model: intracisternal kaolin produces hydrocephalus in fetal lambs and thes
29. Weller RO, Wiśniewski H, Shulman K, Terry RD: Experimental hydrocephalus in young dogs: histological and ultra-
30. Wiśniewski H, Weller RO, Terry RD: Experimental hydro-
cephalus produced by the subarachnoid infusion of silicone oil. J Neurosurg 31:10–14, 1969

Disclosures
The authors report no conflict of interest concerning the materi-
als or methods used in this study or the findings specified in this paper.

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Conception and design: Peiro, Oria, Duru. Acquisition of data: Peiro, Oria, Duru, Scorletti, Vuletin, Encinas, Correa-Martín, Bakri, Sanchez-Margallo. Analysis and interpretation of data: Peiro, Oria, Duru. Drafting the article: Peiro, Oria, Duru. Critical-
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Supplemental Information
Online-Only Content
Supplemental material is available with the online version of the article.

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