Endokrynologia Polska
DOI: 10.5603/EP.2017.0025
Tom/Volume 68; Numer/Number 3/2017
ISSN 0423–104X

Venopunction of the cubital vein as an alternative approach for CGRP plasma level evaluation in tmd patients

Venopunkcja żyły odłokciowej jako alternatywne podejście dla oceny stężenia CGRP w osoczu krwi u pacjentów z dysfunkcją układu ruchowego narządu żucia

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Abstract

Introduction: Calcitonin gene-related peptide is an important vasodilator. It plays an important role in the metabolism of chewing muscles. The aim of the study was to evaluate the plasma level of CGRP in patients with myofascial pain (RDC/TMD Ia) and myofascial pain with limited opening (RDC/TMD Ib) before and after occlusal splint therapy (Michigan splint).
Material and methods: A randomised trial was performed including 39 patients (males = 3, females = 36). Blood samples were taken from the external jugular vein (JUG) and cubital vein (CUB) before and after 30 days of occlusal splint therapy. Plasma levels of CGRP were measured with ELISA KIT for Human Calcitonin Gene Related Peptide (CGRP) 96T (USCNK Business Co. Ltd.).
Results: The results of the study show that the plasma CGRP level was higher in the external jugular vein (JUG1 = 5.07pg/mL [SD = 1.99]) than in cubital vein (CUB1 = 4.3 pg/mL [SD = 1.6]). After 30 days of the occlusal splint therapy the levels in both veins increased: JUG2 = 6.07 pg/mL (SD = 2.19), and CUB2 = 4.9 pg/mL (SD = 1.4). The CGRP plasma level increase was statistically significant only in the external jugular vein (JUG) ($p < 0.05$). Statistically significant pain intensity reduction was observed: VAS1 = 5.4 (SD = 2.08) decreased to VAS2 = 1.7 (SD = 2.07) after splint therapy ($p < 0.05$).
Conclusions: Venepuncture of an external jugular vein is more precise, than venepuncture of a cubital vein in evaluating CGRP plasma level changes in patients with TMD. **(Endokrynol Pol 2017; 68 (3): 326–331)**

Key words: *occlusal splint; external jugular vein; cubital vein; venepuncture; TMD; CGRP*

Streszczenie

Wstęp: Peptyd pochodny genu kalcytoniny jest ważną substancja naczynio-rozkurczową. Odgrywa ważną role w metabolizmie mięśni żucia. Celem pracy była ocena stężenia osoczowego CGRP u pacjentów z bólem mięśniowo-powięziowym (RDC/TMD Ia) oraz z bólem mięśniowo-powięziowym z ograniczonym odwodzeniem (RDC/TMD Ib), przed i po terapii szyną okluzyjną (Szyna Michigan).
Materiał i metody: Przeprowadzono badanie randomizowane, do którego włączono 39 pacjentów (mężczyźni = 3, kobiety = 36). Próbki krwi pobrano z żyły szyjnej zewnętrznej (JUG) oraz z żyły odłokciowej (CUB), przed leczeniem i po 30 dniach stosowania szyny okluzyjnej. Stężenie neuropeptydu CGRP oceniano za pomocą zestawu ELISA KIT for Human Calcitonin Gene Related Peptide (CGRP) 96T (USCNK Business Co. Ltd.).
Wyniki: Stwierdzono, że stężenie neuropeptydu CGRP było wyższe w materiale pobranym z żyły szyjnej zewnętrznej JUG1 = 5,07 pg/ml (SD = 1,99), niż w materiale pobranym z żyły odłokciowej CUB1 = 4,3 pg/ml (SD = 1,6). Po 30 dniach terapii szyną okluzyjną średnie stężenie CGRP w obu grupach CUB i JUG wzrosły: JUG2 = 6,07 pg/ml (SD = 2,19) i CUB2 = 4,9 pg/ml (SD = 1,4). Wzrost stężenia CGRP w osoczu krwi był istotny statystycznie jedynie w materiale pobranym z żyły szyjnej zewnętrznej (JUG) ($p < 0,05$). Zaobserwowano także istotną statystycznie redukcję natężenia dolegliwości bólowych w skali VAS: VAS1 = 5,4 (SD = 2,08) redukcja do VAS2 = 1,7 (SD = 2.07), po przeprowadzonej szynoterapii ($p < 0,05$).
Wnioski: Wenopunkcja żyły szyjnej zewnętrznej dostarcza bardziej precyzyjnych pomiarów stężenia neuropeptydu CGRP w osoczu niż wenopunkcja żyły odłokciowej, u pacjentów z bólową postacią dysfunkcji narządu żucia. **(Endokrynol Pol 2017; 68 (3): 326–331)**

Słowa kluczowe: *szyna okluzyjna, zewnętrzna żyła szyjna, żyła łokciowa, wenopunkcja, TMD, CGRP*

The source of funding was the Silesian Medical University in Katowice, Poland, for the development of the Department of TMD and Orthodontics (KNW-2-017/N/4/K).

Introduction

Calcitonin gene-related peptide is a neuroinflammatory molecule promoting nociceptive and neuroimmune responses [1]. It was recently implicated in TMD (Temporomandibular Disorders) pathophysiology, with chronic muscle pain [2]. CGRP (Calcitonin gene-related peptide) was first discovered by Amara in 1982 in the

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thyroid tissue of aging rats [3]. A human form of CGRP was first isolated from medullary thyroid carcinomas. Two isoforms of CGRP have been discovered (α CGRP, ß CGRP), but the ß CGRP is poorly understood [3]. Human α CGRP is primarily located in unmyelinated, small-diameter sensory C fibres, commonly found in close contact with the vasculature, especially the arterial side. Vasodilator activity of CGRP was first described by Brain in 1985, who characterised it as the most potent microvascular vasodilator [4]. An intradermal injection of CGRP was sufficient to induce erythema and increase blood flow in that area [3]. Small doses injected into human skin produce an erythema that lasts for 5–6 hours [4]. The microvasculature responds strongly to the CGRP: its potency is 10-fold greater than the prostaglandins and 100–1000 times greater than other classic vasodilators. Intravenous CGRP administration causes facial flushing. Higher doses of CGRP cause skin redness, lasting for several hours, and even hypotension. CGRP delivered intravenously causes ionotropic and chronotropic heart effects. The CGRP is released from activated trigeminal sensory nerves, dilates intracranial and extracranial blood vessels, and centrally modulates vascular nociception [5].

Endogenous CGRP promotes tumour-associated angiogenesis and tumour growth. Another recent study supports the cardioprotective role of CGRP against ischaemia/reperfusion injury [6]. Circulating levels of CGRP are reduced in patients with hypertension [7]. After CGRP receptors activation nitric oxide (NO) is synthesised and vasodilation takes place. It was also observed that the effect of CGRP on microcirculation of specific tissues (specific sensitivity) is much more intensive that in the entire circulation. That was the reason for stating the hypothesis in our study: to find out if the CGRP plasma level is the same in the external jugular vein and the cubital vein. The level of this neuropeptide decreases with age. Mice treated with CGRP receptor antagonist presented a decrease in pain duration and pain intensity [2].

This study is a continuation of previous research: "CGRP plasma level changes in patients with temporomandibular disorders, treated with occlusal splints: a randomised clinical trial", performed in 2014. The aim of this study was to evaluate the plasma level changes of CGRP (in patients with TMD/RDC Ia and Ib- Research Diagnostic Criteria for Temporomandibular Disorders, after an occlusal splint therapy) and to compare CGRP plasma levels between the external jugular vein and cubital vein. Cubital vein venepuncture is much easier to perform and less stressful for the patient. If CGRP plasma levels were comparable in both samples, it would be easier and much more comfortable for a patient to participate in an experimental study.

Material and methods

A randomised clinical trial was performed including 105 patients. Of these, 66 patients were excluded for the following reasons: 30 of them did not match inclusion criteria, such as RDC/TMD Ia or Ib diagnosis, bruxism symptoms, or agreement to use a splint [8] — a Michigan splint was produced for the upper arch in each patient; in 26 patients venepuncture of the cubital vein (CUB) or external jugular vein (JUG) was impossible to perform twice (before or after the occlusal splint therapy); and 10 patients did not return for follow-up visits. Thirty-nine patients were enrolled to the study (women = 36 and men = 3), aged 19–70 years (average = 46 years). Patients were examined using the RDC/TMD clinical and physical examination form [8]. One physician enrolled participants in the study and another one assigned them to the interventions. The main inclusion criteria were: positive RDC/TMD Ia or Ib, patient's agreement to participate in the study and to perform two venepunctures. Blood samples were taken from the external jugular vein and cubital vein during the first visit and after 30 days of splint therapy. If the myofascial pain was equal on both sides, we preferred the right jugular vein. Patients were in a supine position on a dental unit chair in the treatment room when the physician performed the venepuncture. The study was approved by the Bioethical Committee of the Silesian Medical University (document number KNW/0022/KB1/104/I/14.)

Exclusion criteria were: anticoagulation treatment, platelet or coagulation disorders, primary headaches, ophthalmological, neurological, or cardiovascular diseases (with hypertension), head traumas in the past six months, secondary headaches, and trigeminal neuralgias.

Myalgia (RDC/TMD Ia) and reduction of mouth opening (RDC/TMD Ib) were indications for preparing occlusal appliances, which patients were given to use during sleep for one month (30 days). Blood samples were collected twice (on the first visit: JUG1, CUB1, and after 30 days of splint therapy: JUG2, CUB2) in each patient, from the external jugular vein (JUG) and cubital vein (CUB).

CGRP plasma levels were measured with ELISA KIT for Human Calcitonin Gene-Related Peptide (CGRP) 96T (USCNK Business Co. Ltd.). Data collected during biochemical analysis were noted in Excel files. The results were analysed with Statistica 7.0 for each group, and Wilcoxon Test analysis was performed ($p = 0.05$).

Results

The results of the study show that the plasma CGRP level was higher in the external jugular vein JUG1 =

5.07 pg/mL than in the cubital vein CUB1 = 4.3 pg/mL, but an increase in CGRP level was observed in each group. After 30 days of the occlusal splint therapy, levels in both veins changed and were elevated: JUG2 = 6.07 pg/mL and CUB2 = 4.9 pg/mL. In the external jugular vein (JUG) an increase of CGRP level was statistically relevant, but in the cubital vein (CUB) it was not statistically relevant. In the present study we provide data suggesting that the CGRP plasma level is comparable in the cubital vein and in the external jugular vein, but higher CGRP levels are marked in the external jugular vein. The venepuncture of an external jugular vein provides blood samples of higher CGRP concentration.

Comparison of CGRP plasma level changes in cubital vein before and after a splint therapy

The CGRP plasma level changes in a cubital vein (Fig. 1) before and after a splint therapy in the experimental group were statistically irrelevant, $p = 0.054$.

Comparison of the CGRP plasma level changes in external jugular vein before and after splint therapy

The CGRP plasma level changes in an external jugular vein (Fig. 2) before and after a splint therapy in experimental group were statistically relevant, $p < 0.05$ ($p = 0.000076$).

Comparison of the pain intensity changes in VAS (Visual analogue scale) before and after a splint therapy

The pain intensity reduction after splint therapy in the experimental group (Fig. 3) was statistically relevant, $p < 0.05$($p = 0.0$).

In both the JUG (external jugular vein) and CUB (cubital vein) an increase in CGRP concentration was observed; it was statistically relevant for the external jugular vein (JUG1 = 5.07 and JUG2 = 6.07 pg/mL). For the cubital vein (CUB1 = 4.3 pg/mL and CUB2 = 4.9 pg/mL) CGRP plasma level changes were statistically insignificant ($p > 0.05$). The pain intensity reduction after splint therapy in the experimental group was statistically significant: VAS1 = 5.4 (SD = 2.08) decreased to VAS2 = 1.7 (SD = 2.07), ($p < 0.05$). The results of our previous study with CGRP are similar. The same tendencies in plasma CGRP levels are observed in both trials.

DISCUSSION

CGRP is released after stimulation of TRPV1 (Transient Receptor Potential Ion Channels) receptors and TRPA (Transient Receptor Potential Ion Channels) receptors, by capsaicin, noxious heat, and most importantly in our

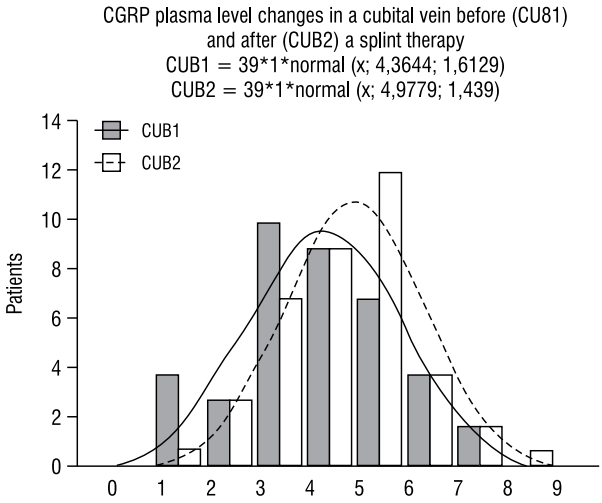

Figure 1. *CGRP plasma level changes in cubital vein before and after splint therapy*

Rycina 1. *Zmiany stężenia osoczowego CGRP, w żyle odłokciowej, przed i po terapii szyną okluzyjną*

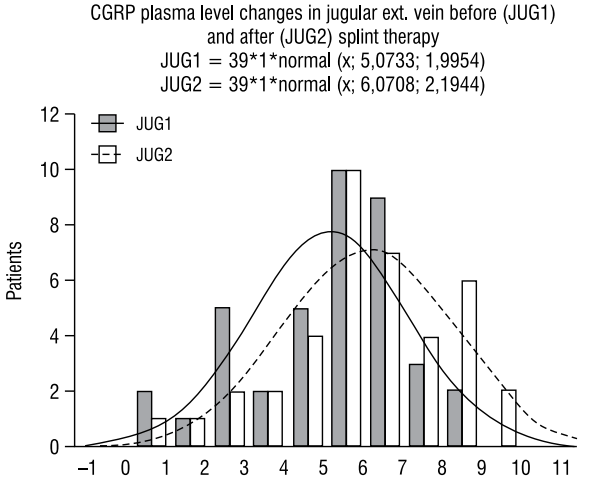

Figure 2. *CGRP plasma level changes in external jugular vein before and after splint therapy*

Rycina 2. *Zmiany stężenia osoczowego CGRP, w żyle szyjnej zewnętrznej, przed i po terapii szyną okluzyjną*

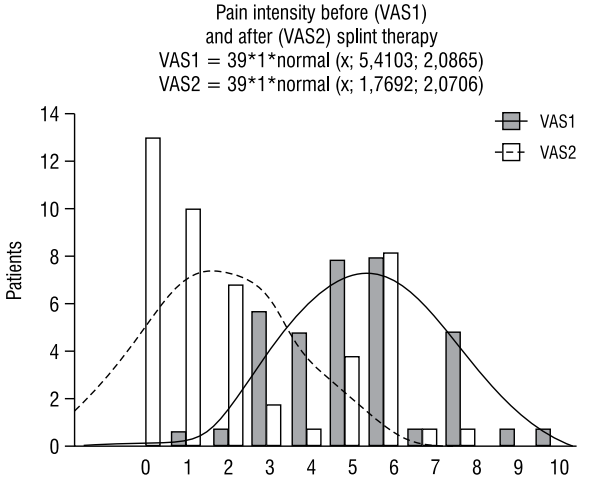

Figure 3. *Pain intensity before and after splint therapy*

Rycina 3. *Nasilenie bólu przed i po terapii szyną okluzyjną*

study, by the high concentration of protons (lowered environmental pH) [7]. Myalgia is often accompanied by anaerobic muscle metabolism and accumulation of lactate. Glycolysis provides anaerobic energy in extremely hard or long lasting muscle activity. The concentration of lactate is rising then, and lowered environmental pH appears [9]. In our study the patients suffered from muscle pain. The CGRP plasma level was expected to be elevated. Average CGRP plasma levels in the present study were: CUB1 = 4.3 pg/mL (1.14 pM), CUB2 = 4.9 pg/mL (1.3 pM) and JUG1 = 5.07 (1.35 pM), JUG2 = 6.07 pg/mL (1.6 pM). Physiological plasma levels of CGRP in humans are usually in the picomolar range, and this low level is attributed to overspill from the release site to systemic circulation. Elevated concentrations of CGRP are observed in patients with migraine (345 pg/mL = 92 pM), comparing to healthy patients (150 pg/mL = 40 pM). Cernuda-Morollon determined CGRP plasma levels in patients with migraine in the right antecubital vein using an ELISA kit. In patients with chronic migraine the average CGRP levels were 74.9 pg/mL, compared to healthy women 33.74 pg/mL. In patients with episodic migraine 46.37 pg/mL and with cluster headache 45.87 pg/mL [10]. Those levels of CGRP were higher, compared to our study. Sarchielli also confirmed previous findings of an increase in calcitonin gene-related peptide in internal jugular venous blood of migraine without aura [11]. In Reynaud's Phenomenon there is a decreased level of CGRP in digital circulation. Patients receiving CGRP intravenously at room temperature have a proper digital vasoconstriction. At 5°C the effect of CGRP administration is poor [3].

Appelgren reported the important role of CGRP in TMD in 1995 [12]. Vause and Durham stated that CGRP is released from neurons of the trigeminovascular system and promotes trigeminal ganglion inflammation, which mediates peripheral sensitisation and temporomandibular disorders [13]. Kopp reported the connection between an anterior open bite and high levels of CGRP [14].

There are studies comparing CGRP levels in the external jugular vein and the radial or cubital vein [15, 16]. The aim of the study was evaluation of local and peripheral CGRP plasma concentration in patients with TMD. Blood samples collected from the cubital vein were less useful than those from the external jugular vein because of the lower CGRP level. Ashina and Bendtsen also observed elevated CGRP levels in blood collected from the cubital vein [15]. Tvedskov and Ashina found no difference between CGRP levels in the cubital vein and the external jugular vein in patients with migraines [16]. Frieberg compared CGRP and other neuropeptides levels in migraineurs by repeated blood samples obtained from the carotid artery and the internal jugular vein and found no detectable changes. All migraineurs had an overall elevated mean CGRP value compared to control values [17]. The ELISA KIT used in our study was sensitive enough to detect CGRP in the jugular vein and in the cubital vein in TMD patients. Elevated CGRP plasma levels, in external jugular vein and in cubital vein, were found after 30 days with occlusal splints, accompanied by pain relief. A statistically significant reduction in pain intensity based on the VAS scale was observed and patients' health improved as well. Venepuncture was one of our trial limitations because the procedure was stressful for the patients, and not every patient decided to participate in the trial. Another limitation was the short period of time between the collection of the first and second blood samples. The samples used for measurements can only be frozen at –70°C for one month. In 1999, Parlapiano evaluated CGRP plasma levels and ET-1 plasma levels in normal subjects and reported that the mean plasma CGRP level was 42.8 pg/mL. The plasma concentration was measured using a radioimmunoassay kit (Peninsula Labs), but the authors did not mention which blood vessels the blood samples were collected from [18]. Elevated levels of CGRP were also analysed by Joyce, who found that in healthy patients, the level was 2.0 ± 0.3 pg/mL and that in patients with sepsis, the level was 14.9 ± 3.2 pg/mL [19]. CGRP plasma levels in this study are similar to our results. These findings contribute to a decreased vascular resistance in dilatory states and increased cardiac output in septic states. The same vascular dilatation may occur in masticatory muscles of patients with TMD.

Muscle pain causes release of neuropeptides, which initiate and maintain neurogenic inflammation. We know that 5-HT (5-hydroxytryptamine, serotonin) and PGE2 (prostaglandin 2 receptor) are involved in the development of pain and hyperalgesia or allodynia of the masseter muscle in patients with fibromyalgia, whereas myofascial pain seems to be modulated by other, as yet unknown, mediators. Interaction between the peripheral nervous system, the immune system, and local cells is probably of great importance for the modulation of pain and inflammation in the TMJ (temporomandibular joint) and orofacial musculature [14]. Peripheral and central sensitisation leads to hyperalgesia and allodynia. Cady injected CGRP into the TMJ capsule of rats and observed the inflammation process and sensitisation [20]. Sato concluded that CGRP may play an important role in the pain mechanism and that CGRP elevated levels may be correlated with joint pain [21]. Bick demonstrated that CGRP causes calcium mobilisation in skeletal muscle cells. High levels of CGRP caused continuous tetanus, which could be the reason for muscle-tension headaches. Bick conducted different studies with various aspects of muscle sore-

Table I. Median values of the CGRP plasma level before (1) and after 30 days (2) of occlusal splint therapy

Tabela I. Średnie wartości osoczowego stężenia CGRP przed (1) oraz 30 dni po (2) szynoterapii szyną okluzyjną

	JUG V.	CUB V.	VAS
Before a splint therapy (1)	5,07 pg/mL	4,3 pg/mL	VAS1 = 5,4
After a splint therapy (2)	6,07 pg/mL:	4,9 pg/mL	VAS2 = 1,7

ness, exercise, and repair [22]. Homonko observed an increased number of CGRP+ motoneurons in muscles after physiological neuromuscular activity [23, 24]. The exercise regimen may result in damage to the muscle tissue, thereby initiating repair and regenerative mechanisms [25]. This repair process may be the reason for the elevated concentration of CGRP in our experimental group of patients with TMD. Jonhagen detected increased CGRP levels after eccentric exercise in healthy subjects, which may reflect tissue regeneration [24]. Eccentric contraction can occur after muscles are stretched during contraction [25]. CGRP muscle levels were estimated in vivo by microdialysis followed by radioimmunoassay and were found to be increased a few days after eccentric exercise, and this may also be the reason for the elevated CGRP plasma levels in our experimental group of patients. The eccentric exercise performed in the Jonhagen study may be similar to the masseter and temporalis muscle exercises with occlusal splints in our experimental group [24]. According to Dessem the masseter muscle has a limited ability to repair itself after injury and a tendency to undergo apoptosis [26]. Dessem also presented evidence that masticatory muscles do not adapt to repeated injury (bruxism), such as that occurring in hindlimb muscles; therefore, masticatory muscles are more susceptible to injuries and chronic muscle pain.

Mc Daniel noticed that blood flow in muscles is altered by changes in muscle length: the longer and more relaxed the muscle is, the higher the blood flow [27]. Occlusal splints change the vertical dimension and stretch masseter and temporal muscles. The altered muscle length decreases muscle tone and produces masticatory muscle relaxation, which is probably the reason for increased muscular blood flow. These findings suggest that CGRP may participate in muscle contraction and stretching. Cerebral blood vessels are innervated by sensory nerves that store several neurotransmitters. In primary headaches there is a clear association between head pain and the release of the neuropeptide CGRP [28–31]. Muscle spasm may also be treated successfully with botulinum toxin type A(BTX-A) by local muscle injection, as reported by Guarda-Nardini in an investigation of its efficacy in reducing myofascial pain symptoms in bruxers [32]. Muscle stretching with occlusal splints increases CGRP release from the trigeminovascular system, but BTX-A produces muscle paralysis and decreases the CGRP concentration. An interesting observation in our study is that during the time of elevated CGRP plasma concentration, there was a negative correlation between the VAS scale and the CGRP plasma level concentration. A similar situation was observed by Ashina, who indicated that ongoing activity in sensory neurons in cranial muscles may result in plasma CGRP level changes in patients with chronic headache. Eight patients had higher CGRP plasma levels in the delayed headache-free period. However, a small number of patients were enrolled in this study, and the CGRP plasma levels in the cranial and peripheral circulation may be undetectable [3]. Eccentric muscle contraction and rapid stretching produces scattered myofibres and intramuscular plasma extravasation [25]. CGRP vasodilates blood vessels in muscle tissue and mediates neurogenic inflammation. In his study Donnerer noted an important regulatory function of neuropeptides such as CGRP and substance P in healing processes [33].

Conclusions

The CGRP plasma level is increased in the external jugular vein and in the cubital vein after 30 days of splint therapy in patients with TMD. The mechanism of CGRP accumulation is not well known. In our study the plasma level of CGRP changed in both sample collections (JUG and CUB), but it was statistically relevant only in samples collected from the external jugular vein (JUG). The concentration of neuropeptide CGRP is detectable in the cubital vein, but the increase that we observed was not statistically important. For more precise results, it is better to collect samples from the external jugular vein rather than from the cubital vein. This neuropeptide is probably implicated in muscle healing and may serve multiple functions, including muscle repair. This study shows that CGRP is involved in muscle contractility and the myofibre repair process in patients with TMD.

Acknowledgements

We would like to acknowledge Piotr Buchta, Krzysztof Nitecki, and Teresa Ogonowska for their technical support. The authors have no conflict of interest regarding this commentary.

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