

## Review Article

# Calcium, Channels, Intracellular Signaling and Autoimmunity<sup>☆</sup>

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### ARTICLE INFO

#### Article history:

Received 31 March 2013

Accepted 8 May 2013

Available online 21 December 2013

#### Keywords:

Calcium signals

T lymphocyte

B lymphocyte

Store-operated calcium entry

Calcium-release-activated calcium

Stromal interaction molecule

Orai1

Autoimmune diseases

### ABSTRACT

Calcium ( $\text{Ca}^{2+}$ ) is an important cation able to function as a second messenger in different cells of the immune system, particularly in B and T lymphocytes, macrophages, and mastocytes, among others. Recent discoveries related to the entry of  $\text{Ca}^{2+}$  through the store-operated calcium entry (SOCE) have opened a new investigation area about the cell destiny regulated by  $\text{Ca}^{2+}$  especially in B and T lymphocytes. SOCE acts through calcium-release-activated calcium (CRAC) channels. The function of CRAC depends upon two recently discovered regulators: the  $\text{Ca}^{2+}$  sensor in the endoplasmic reticulum or stromal interaction molecule (STIM-1) and one subunit of CRAC channels called Orai1.

This review focuses on the role of  $\text{Ca}^{2+}$  signals in B and T lymphocytes functions, the signaling pathways leading to  $\text{Ca}^{2+}$  influx, and the relationship between  $\text{Ca}^{2+}$  signals and autoimmune diseases.

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#### Palabras clave:

Calcio

Linfocito T

Linfocito B

Store-operated calcium entry

Calcium-release-activated calcium

Stromal interaction molecule

Orai1

Enfermedades autoinmunes

## Calcio, canales, señalización intracelular y autoinmunidad

### RESUMEN

El calcio ( $\text{Ca}^{2+}$ ) es un catión con capacidad multifuncional como segundo mensajero en diferentes grupos celulares del sistema inmunitario que incluyen los linfocitos T y B, los macrófagos, los mastocitos, entre otras. Los recientes descubrimientos en relación con la entrada de  $\text{Ca}^{2+}$  dependiente de depósito (SOCE por su sigla en inglés, *store operated calcium entry*) han abierto nuevos caminos en la investigación de cómo este catión dirige el destino celular, en especial en los linfocitos T y B. La SOCE actúa a través de canales CRAC (del inglés *Ca<sup>2+</sup> release-activated Ca<sup>2+</sup> channels*) y su mecanismo de activación depende de la interacción de dos moléculas reguladoras: un sensor del  $\text{Ca}^{2+}$  del retículo endoplásmico o molécula de interacción estromal (STIM-1, del inglés *stromal interaction molecule*) y una subunidad poro del canal CRAC (Orai1).

Esta revisión se centra principalmente en las funciones del  $\text{Ca}^{2+}$  en los linfocitos B y T, así como las alteraciones de estas vías implicadas en el desarrollo de enfermedades autoinmunes.

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### Overview

Calcium ( $\text{Ca}^{2+}$ ) is a multifunctional cation capable of acting as a second messenger in various immune cell groups including T and B lymphocytes, macrophages, mast cells, etc.<sup>1–3</sup> Its distribution in intra- and extracellular spaces makes specialized pumps and channels necessary for its functioning and mobilization, as well as the

influence of the cell depolarization or repolarization. Furthermore, the amount and duration of the flow of  $\text{Ca}^{2+}$  will determine the type and duration of its effect on intracellular signaling. Recent discoveries in connection with the reservoir dependent  $\text{Ca}^{2+}$  entry (SOCE by its acronym) *have broken new ground in the research of how this cation directs cell fate, especially in T cells and B. This review focuses primarily on the roles of  $\text{Ca}^{2+}$  in the last 2 cell groups and their involvement in autoimmunity.*

### Calcium as an Intracellular Signaling Element

For normal cell function, especially regarding the immune system, the starting point is a basic principle consisting in the presence of the following elements:

<sup>☆</sup> Please cite this article as: Izquierdo JH, Bonilla-Abadía F, Cañas CA, Tobón GJ. Calcio, canales, señalización intracelular y autoinmunidad. Reumatol Clin. 2014;10:43–47.

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- An external signal.
- A receptor.
- An internal signal, which depends on turn on the modifications undergone by the receptor in contact with the external signal and the cofactors that amplify the received signal.
- A transcription factor which moves to the nucleus.
- Transcription of ribonucleic acid (RNA) and translation to generate the protein(s) induced by the external signal.

Molecular biology studies and recent descriptions of<sup>4</sup> new proteins have advanced the understanding of how these components of cell signaling work. External signals are formed by different proteins or peptide derivatives (cytokines, chemokines, pathogen associated peptides, etc.) which are picked up by a receptor located on the cell membrane. In the case of T and B lymphocytes, these receptors require the participation of other elements or co-receptors, which allow adequate extracellular signal synapses in order to activate the intracellular signaling cascade. The internal signal (or second messenger) is generated by multiple mechanisms (usually phosphorylation and dephosphorylation) through different routes, including JAK-STAT pathway MAP kinases, G-protein pathways, etc.<sup>5</sup>

Ca<sup>2+</sup> acts as a second messenger and its importance is becoming more known thanks to recent findings regarding SOCE and its implications on the durability of the cellular responses to Ca<sup>2+</sup> flux. Ca<sup>2+</sup>-induced signals were known for several decades, mainly due to their importance in RNA synthesis and cell division in leukocytes and thymocytes.<sup>6,7</sup> Currently, there are various known functions dependent on the amount of Ca<sup>2+</sup> present at the intracellular level. When Ca<sup>2+</sup> levels increase during a short period they reduce lymphocyte mobility, thus enhancing the immunological synapse. In the case of other cell groups, high levels of short lived Ca<sup>2+</sup> lead to other phenomena such as T cell-mediated cytotoxicity, release of lytic granules and/or cellular recognition processes as well as apoptosis. In contrast, when Ca<sup>2+</sup> levels rise for a prolonged period, they can regulate transcriptional responses that will mark the cell fate of T and B lymphocytes (e.g. Cytokine production, cell differentiation, effector functions, non responsive states, etc.).<sup>8</sup> These Ca<sup>2+</sup> levels in lymphocytes are regulated dynamically through various channels, among which we find<sup>9</sup>:

- Intracellular channel receptors and the receptor for inositol-1,4,5-triphosphate (IP3) located in the sarcoplasmic reticulum.
- Plasma membrane channels, including Ca<sup>2+</sup> release-activated Ca<sup>2+</sup> channels (CRAC).
- Ca<sup>2+</sup>-dependent potassium channels activated by voltage.
- Energy dependent transporters channels.
- Nonselective cation channels.
- The sarco-endoplasmic reticulum adenosine triphosphatase pump (SERCA) and plasma membrane Ca<sup>2+</sup> ATPase (PMCA).

In addition to these channels, the same concentration of intracellular Ca<sup>2+</sup> entry helps regulate CRAC ion channels and the IP3 pathway, as well as the state of cell membrane repolarization. Once you increase the intracellular levels of Ca<sup>2+</sup>, this activates other signaling cascades that define the lymphocyte cell fate as reviewed below.

### Calcium and Signaling in Lymphocytes

Once the T or B lymphocyte is activated by its receptor and the process of costimulation occurs, recruitment, and activation of a group of protein tyrosine kinases, bound to other adapter proteins lead to the phosphorylation and activation of phospholipase C- $\gamma$  (PLC  $\gamma$  1  $\gamma$  PLC in T and B lymphocytes 2). This enzyme, in turn,

hydrolyzes phosphatidylinositol-3,4-bisphosphate (PIP2) from the cell membrane to 2 second messengers:

- IP3.
- Diacylglycerol (DAG).

IP3 then shifts and binds to its receptor on the membrane of the endoplasmic reticulum (ER) causing the release of Ca<sup>2+</sup> stored inside and activation of other intracellular signals (Figs. 1 and 2). However, the rapid depletion of the ER Ca<sup>2+</sup> leads to a short response. To facilitate the extension of cellular responses, another route of entry of Ca<sup>2+</sup>, called SOCE, is activated, which was recently discovered by large-scale searches of RNA interference. SOCE acts through CRAC channels and its mechanism of activation depends on the interaction of two regulatory molecules<sup>10</sup>:

- A Ca<sup>2+</sup> sensor in the ER or stromal interaction molecule (STIM-1).
- A CRAC channel pore subunit (Orai1).

The operation of this mechanism has been described thus: once the Ca<sup>2+</sup> sensing STIM detects the decrease in the concentration of Ca<sup>2+</sup> into the ER, it undergoes a conformational change (oligomerization and aggregation) forming a “point”, in juxtaposition to CRAC located as a transmembrane channel. This conformation makes contact with the Orai1 pore channel subunit, allowing the opening of the channel and the entrance of Ca<sup>2+</sup>. This increases the intracellular levels of the ion and perpetuates the responses generated by the activation of lymphocytes and their respective receptors. Other mechanisms have been described for regulating CRAC channel activity, including the activity of other Ca<sup>2+</sup>-permeable channels such as the TRPC family members, but this mechanism is not entirely clear. Also, other mechanisms have been postulated as regulators of STIM-Orai1 binding, where the cytoskeleton and the negatively charged membrane phospholipids can be an influence.<sup>11</sup>

### Signaling Pathways Activated After Calcium Influx

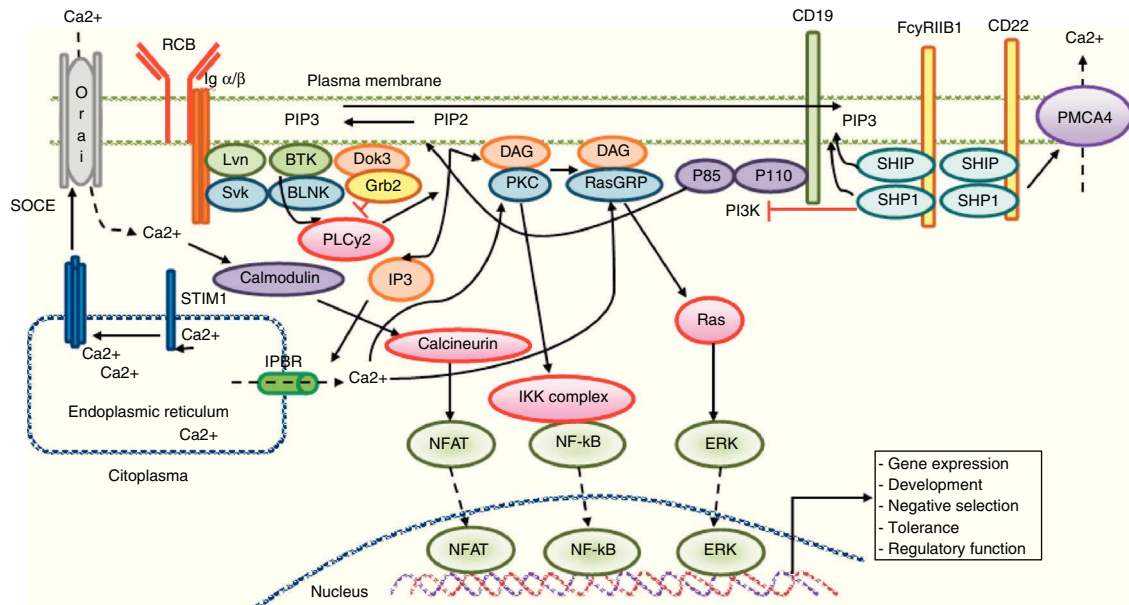
Once the increase in intracytoplasmic Ca<sup>2+</sup> levels is achieved, they activate other signaling pathways and transcription factors that bind DNA and finally lead to the production of proteins, cytokines, etc. related to the inflammatory cascade. Within these pathways we find<sup>12-14</sup>:

- The calmodulin–calcineurin pathway, with the final activation of the nuclear factor of activated T cells (NFAT).
- The Ca<sup>2+</sup>-dependent kinase-calmodulin (CaMK) pathway, which has as transcription factors a protein bound to a response element of cyclic adenosine monophosphate (CREB, cyclic-adenosine monophosphate-responsive element binding protein) and myocyte enhancer factor 2.
- The nuclear factor  $\kappa$ B (NF- $\kappa$ B).

Upon hydrolysis of PIP2, DAG is also obtained, which in turn can activate an additional two signaling pathways:

- The protein kinase C pathway (PKC), and the Ras-mitogen-activated protein kinase.
- The protein kinase C (PKC) pathway.

These pathways ultimately activate transcription factors such as AP-2 (a transcriptional complex formed by c-Fos and c-Jun) and NF-B.  $\kappa$  The various components and pathways related to the flow of Ca<sup>2+</sup> are summarized in Table 1.

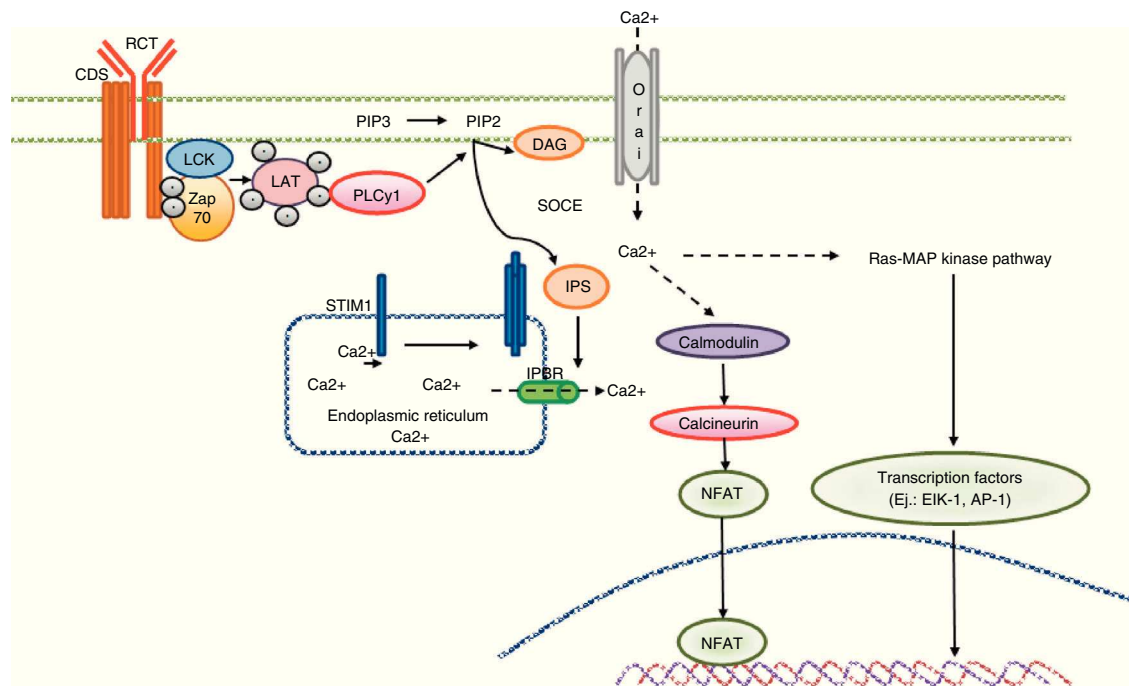


**Figure 1.** Calcium signaling in B lymphocyte. Antigen recognition by the B cell receptor activates different protein kinases, such as Lyn and Syk BLNK, leading to the activation of PLC  $\gamma$  2, which hydrolyzes membrane bound PIP2 into small amounts of DAG and IP3. IP3 binds to its receptor IP3R located in the endoplasmic reticulum membrane and allows the release of stored calcium. Subsequently, the decrease in calcium level into the lumen of the endoplasmic reticulum generates the translocation of STIM1, which leads to the opening of the calcium channel to induce Orai1 dependent calcium entry from reservoir (SOCE). The CD19 receptor facilitates activation of the PI3K p110 subunit. This enzyme phosphorylates PIP2 to produce PIP3. The Fc receptor to low affinity IgG (Fc $\gamma$ RIIB1) and CD22 are both negative regulators of calcium signaling during stimulation of B-cell receptor. The negative function of these receptors is mediated by SHIP and SHP1. Additionally, CD22 inhibits signaling through calcium efflux mediated by PMCA4. Calcium entry generates activation of transcription factors responsible for the induction of new proteins responsible for the functions of proliferation, differentiation, and immune response.

**Calcium and Autoimmunity**

Signaling through intracellular Ca<sup>2+</sup> has been implicated in the pathogenesis of autoimmune and congenital immunodeficiencies. Diseases such as systemic lupus erythematosus (SLE), rheumatoid

arthritis (RA), multiple sclerosis (MS) and type 1 diabetes mellitus are related in their pathophysiology by the presence of autoreactive T and B cells that contribute in the generation of the inflammatory process. In SLE, autoreactive T cells replace the X $\Delta$ 3- $\zeta$  chain with a  $\Phi$  $\chi$ P- $\gamma$  common chain, leading to an intracellular signaling



**Figure 2.** Calcium signaling in the T cell. Antigen recognition by the T cell receptor leads to tyrosine kinase activation inside the T cell, such as LCK and ZAP70, which results in phosphorylation and activation of  $\gamma$  PLC 1. PIP2 latter hydrolyzed in IP3 and DAG. The IP3 receptor opens the IP3R located in the endoplasmic reticulum and allows the output of calcium from the endoplasmic deposits. Calcium sensors detect STIM2 STIM1 and reducing calcium deposits by the N-terminal region of the lumen of the endoplasmic reticulum. STIM proteins are added in small groups in the endoplasmic reticulum membrane and produce extracellular calcium entry via the CRAC channel, Orai1. Intracellular calcium concentration activates the calcineurin pathway, NFAT, and the Ras-MAP kinase pathway.

**Table 1**  
Calcium and Cell Function in B and T Lymphocytes.

Molecule	Calcium signal	Cell biology
BTK	Increases output of Ca <sup>2+</sup> from ER	Early B lymphocyte development, immune response, immune function loss
BLNK (SLP-65)	Increases output of Ca <sup>2+</sup> from ER	Early B lymphocyte development, immune response, immune function loss
PLC $\gamma$ 2	Increases output of Ca <sup>2+</sup> from ER	Early B lymphocyte development, immune response, autoimmunity by gain of function
PLC $\gamma$ 1	Increases output of Ca <sup>2+</sup> from ER	T lymphocyte development, immune response, immunodeficiency due to loss of function
CD19	Increases output of Ca <sup>2+</sup> from ER	B lymphocyte development, immunodeficiency due to loss of function
SHP1	Inhibits Ca <sup>2+</sup> increase	Autoimmunity by loss of function
SHIP	Inhibits Ca <sup>2+</sup> increase	B lymphocyte development
CD22	Inhibits Ca <sup>2+</sup> increase	Autoimmunity by loss of function
$\gamma$ Fc RIIB	Inhibits Ca <sup>2+</sup> increase	Autoimmunity by loss of function
ITPKb	Inhibits SOCE	T lymphocyte development, immune response
Grb2	Inhibits (IgM B lymphocytes) or increases (IgG B lymphocytes) Ca <sup>2+</sup> output	B lymphocyte development, immune response, production of autoantibodies by loss of function
STIM-1/2	Increases SOCE	Suppression of autoimmune inflammation
Zap70	Increases output of Ca <sup>2+</sup> from ER	T cell activation, gene expression
LCK	Increases output of Ca <sup>2+</sup> from ER	T cell activation, gene expression

via the Syk (spleen tyrosine kinase) pathway and not ZAP-70, as is usual. This, associated with a group of lipid rafts in the plasma membrane of the T cell, increases intracellular Ca<sup>2+</sup> in response to activation of the T cell receptor (TCR) by an autoantigen. The PKC pathway via calmodulin-dependent (CAMK4) is activated, which binds to CREB and increases the production of interleukin 17 (IL-17) at the expense of the production of IL-2.<sup>15</sup> This phenomenon has also been described in diseases such as RA and MS. However, in these conditions the proposed hypothesis is that the autoreactivity of the T cells due to mutations in the ZAP-70 kinase, markedly reduces TCR dependent signaling, including Ca<sup>2+</sup> signals, allowing the autoreactive TCR to 'escape' negative selection in the thymus but become arthritogenic in peripheral tissues. This raises the issue of Ca<sup>2+</sup> signaling participating in the selection process of thymic T cells.<sup>16</sup> B cells, in the absence or lack of response to SHP1, CD22 or Fc  $\gamma$  RIIB1 generate increased responses to the influx of Ca<sup>2+</sup> and, therefore, B lymphocyte hyperactivity and autoimmunity. In SLE, Fc  $\gamma$  RIIB1 defects are associated with increased response to Ca<sup>2+</sup> in B lymphocytes.<sup>17</sup> Although diseases such as SLE are characterized by a systemic component, alterations in calcium signaling have been described in T and B lymphocytes, and have not been associated with susceptibility to specific manifestations of the disease (e.g. increased risk of nephritis). However, derived from the murine *Orai<sup>K1/K1</sup>* model (discussed below), the lack of calcium signaling in immune cells protects against the development of colitis in the transfer model of inflammatory bowel disease.

As mentioned previously, in T cells, the Ca<sup>2+</sup> entry following antigenic stimulation is essential for the activation of NFAT. It has been demonstrated, in a group of patients with mutations in either STIM 1 or Orai1, that the affected protein function or expression results in a defect of stored Ca<sup>2+</sup> influx and CRAC channel function, resulting in decreased lymphocyte activation. These mechanisms lead to the presentation of an inherited form of severe combined immunodeficiency, demonstrating the importance of these channels in the normal function of T-lymphocytes in humans. Similarly, calcium signaling defect can lead to autoreactive T cells to a state of activation which can result in the development of autoimmune diseases. Given the role of calcium in the activation of autoimmune phenomena, Lin et al. have recently published the generation of humanized monoclonal antibodies directed against high affinity human Orai1. These antibodies showed decreased Ca<sup>2+</sup> entry, NFAT transcription and release of cytokines and may represent a new therapeutic target in the treatment of autoimmune diseases.<sup>18</sup> This

approach affects different tissues involving calcium signaling, and different immune system effects should be better understood.

In an animal model developed by McCarl et al. the role of Orai1 was described in autoimmunity. In this study, T and B lymphocytes derived from a mouse model of *Orai<sup>K1/K1</sup>* expressed a mutated non-functional Orai1 protein. These cells showed a severe decrease in the influx of Ca<sup>2+</sup> and CRAC channel function, resulting in decreased expression of several cytokines, including IL-1, IL-4, IL-17, interferon gamma and tumor necrosis factor (TNF) alpha in CD4 and CD8 T lymphocytes. This model showed greater tolerance to skin grafts compared with animals without the mutation and failed to develop colitis type autoimmune phenomena. These findings confirm the importance of these channels and the influx of Ca<sup>2+</sup> on the activation of the adaptive immune system cells.<sup>19</sup>

Ca<sup>2+</sup> is also involved in the development of congenital immunodeficiencies. In common severe immunodeficiency, for example, there is a mutation in the T cell, more specifically in the transmembrane domain of Orai1 in CRAC, which allows the entry of Ca<sup>2+</sup> via SOCE, giving rise to T lymphocytes with low proliferative capacity and cytokine production. This defect may also affect SOCE B cells and fibroblasts in this disease.<sup>20</sup> In X-linked gammaglobulinemia presents an inherited deficit in B cells, which is characterized by mutations in Bruton tyrosine kinase, the enzyme responsible for the activation of PLC  $\gamma$  2 with the effect of reducing the Ca<sup>2+</sup> entry pathway mediated by SOCE due to<sup>21</sup> low IP3 generation. Finally, common variable immunodeficiency also presented alterations in the functioning of B lymphocytes, especially mutations in CD19, decreasing Ca<sup>2+</sup> influx into the cell, compromising the response to antigens and reducing CD27 positive B cell generation.<sup>22</sup>

## Conclusions

Intracellular Ca<sup>2+</sup> signaling is a component of signaling cascades that are generated in different cells after exposure of its receptors to a specific stimulus. In the case of T and B lymphocytes, these signals give rise to multiple changes in intracellular DNA expression, with the consequent production of various inflammatory markers. Research in this area also has established the importance of Ca<sup>2+</sup> pathways in the development of autoimmune diseases, both due to overproduction as well as inactivity. As progress is made in the study of all these concepts, it becomes important to consider intervention on these pathways and receptors for therapeutic purposes.

## Ethical Responsibilities

**Protection of people and animals.** The authors declare that experiments have not been performed on humans or animals.

**Data confidentiality.** The authors state that no patient data appear in this article.

**Right to privacy and informed consent.** The authors state that no patient data appear in this article.

## Conflict of Interest

The authors declare no conflict of interest.

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