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REVIEW ARTICLE

Angiopoietins in inflammation and their implication in the development of inflammatory bowel disease. A review☆

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Abstract

Background: Angiopoietins are essential angiogenic mediators. Since inflammatory bowel disease (IBD) involves inflammation, ulceration and regeneration of the intestinal mucosa, the angiopoietin system has been proposed as a factor to maintain pathological angiogenesis during the development of the IBD.
Aim: To review the potential role of angiopoietins in the inflammation driven by angiogenesis during the course of the IBD.
Methods: Publications were identified by PubMed searches using the following key words: angiopoietin; Tie-2 receptor; angiogenesis; inflammatory bowel disease and inflammation, in various combinations.
Results: Angiopoietin-1 acts as a regulator of blood vessel maturation and has anti-inflammatory properties, whereas angiopoietin-2 marks the onset of angiogenesis and is required for normal formation of lymph vessels. Both angiopoietins make use of their angiogenic regulatory effects via the angiopoietin tyrosine-kinase receptor (Tie-2). While angiogenesis has been shown to promote and sustain many events of inflammation, the involvement of the angiopoietin system in IBD has been reported in few studies. It is not clear whether the angiopoietins' role in the development of intestinal inflammation is due to an imbalance in the levels of these proteins or this system exerts its pro-angiogenic properties through a different mechanism during the close-loop relationship between angiogenesis and inflammation.

Abbreviations: IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; Tie-2, angiopoietin tyrosine-kinase receptor; VEGF, vascular endothelial growth factor; EC, endothelial cell; VSMCs, vascular smooth muscle cells

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Conclusions: Angiopoietins have key functions in the angiogenic process, and their abnormal activation might depend on their surrounding inflamed environment. The determination of these angiogenic factors in serum and tissue could be useful for monitoring IBD progression.

Contents

1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder that encompasses two different clinical entities: Crohn's disease (CD) and ulcerative colitis (UC) [1,2]. CD may affect any part of the gut from the mouth to the anus, though it is usually located in the terminal ileum and colon. It is characterized by macroscopic affectation and segmental distribution along the gut. On the other hand, UC affects the mucosa of the colon with variable extension from the rectum to the cecum on a continuous basis.

The etiology of CD and UC remains unclear, but it is characterized by inflammation located in the gut due to an altered immune response. Genetic and environmental factors in the development of both diseases are known to be involved [3,4].

During the clinical course of both conditions, flare-ups in activity, characterized by an increase and exacerbation of inflammation, alternate with remission periods. The number, frequency and severity of these flares are unpredictable. Recent investigations have been conducted to elucidate the etiology of these diseases and the factors that might influence their evolution.

For instance, IBD is associated with extensive tissue injury and lymphatic remodeling caused by tissue edema, inflammatory cell infiltrates, numerical or functional alteration of ertain subpopulations of immune cells, loss of epithelial integrity and increased angiogenesis. These features, together with the release of cytokines in the intestinal mucosa, might contribute to the pathogenesis and development of IBD by triggering diverse molecular mechanisms [5–7]. Recently, scientific evidence suggests that vascular development, particularly lymphangiogenesis and angiogenesis, could play a main role as a cause of IBD tissue injury and not simply an epiphenomenon ascribed to inflammation [5,8–10].

2. Methods

Bibliographical searches were performed in PubMed from the earliest records to February 2012 using the following key words (all fields): (angiopoietin OR Tie-2 receptor OR angiogenesis) AND (inflammatory bowel disease OR inflammation). The references from the articles selected for the study were also examined in search of articles meeting the inclusion criteria. Relevant abstracts and other material from meetings were investigated. Studies on angiopoietin function in other diseases were included if relevant information was reported.

2.1. Angiogenesis

Blood vessels originate through two processes called vasculogenesis and angiogenesis. Vasculogenesis starts in the embryonic period from multipotent progenitor cells, while in angiogenesis the vascular networks are created from the pre-existing ones. Physiological angiogenesis takes place during processes like the menstrual cycle, embryonic development, tissue repair and bone growth.[11,12] Angiogenesis is considered to be activated primarily by hypoxia [7,13]. Afterwards, the basal membrane is degraded by metalloproteases and endothelial cells (ECs) proliferate, triggered by the released integrins and adhesion molecules. Finally, pericyte recruitment stabilizes the newly-formed vessels. Upon stabilization and structuring of the new microvessel network, the balance between proangiogenic and antiangiogenic factors in physiological angiogenesis returns to the baseline levels and the

process concludes. However, the level of proangiogenic mediators in pathologic angiogenesis remains augmented and formation of new vessels persists [14]. Altered angiogenesis has been reported in other inflammatory diseases such as cancer, atherosclerosis, ischemic heart disease, rheumatoid arthritis or asthma [12,15,16].

2.2. Cytokines and angiogenesis

As a result of the reciprocal angiogenesis–inflammation activation processes, there is an increase of proinflammatory cytokines, chemokines, eicosanoids, thromboxanes and free radicals in the interstitial medium. Among these released proteins, the most important angiogenic mediator, VEGF-A, has recently gained special relevance. For instance, increased levels of VEGF-A released during active episodes of IBD increase endothelial expression of intercellular adhesion molecule-1 to promote enhanced leukocyte adhesion [17]. Additional VEGF-A action increases blood vascular permeability which intensifies gut edema, a central characteristic of IBD. Finally, new blood vessels formed in the inflamed gut in response to VEGF-A may exhibit delayed maturation, displaying incomplete recruitment of pericytes necessary to stabilize vessels, inhibit endothelial proliferation and reduce vascular leakage [18,19]. Besides the classic angiogenic factors such as VEGF-A, the angiopoietin Ang/Tie system plays an important and complementary role in the regression, maturation and stabilization of blood vessels [20,21].

2.3. Angiopoietin family

Ang-1, Ang-2 and the interspecies orthologs such as Ang-3 (mouse)/Ang-4 (human) are natural ligands of the Tie-2 receptor, which is expressed primarily on ECs and early hematopoietic cells. The other kinase receptor, Tie-1, is an orphan receptor that may also act as an angiopoietin receptor, possibly in complex with Tie-2, but little is known about its function in angiogenesis [22–29]. Angiopoietins do not belong to the VEGF family, but are also key mediators in angiogenesis. The Ang/Tie receptor signaling cascades are involved in fundamental angiogenesis events including vascular stabilization and remodeling, as well as recruitment of pericytes and vascular smooth muscle cells (VSMCs). Ang-1 acts as a regulator of blood vessel maturation and has anti-inflammatory properties. Its stimulation in lymphatic ECs promotes up-regulation of a VEGF receptor – VEGFR-3 – suggesting that it is also indirectly involved in VEGF-C/VEGFR-3 pathway (Fig. 1). On the other hand, Ang-2 is particularly responsible for the initiation of angiogenesis and it is necessary for the normal formation of lymphatic vasculature [25,28,30–34].

2.4. Angiopoietin-1

The first angiopoietin that was discovered was Ang-1 which is secreted by pericytes, mesenchyme and VSMCs of the developing vasculature and it is thought that it stabilizes the formation of newly formed blood vessels [25,35].

Ang-1 promotes EC cell stability by inducing Tie-2 phosphorylation (p-Tie-2) and suppressing endothelial migration, which enhances survival [25,36–42]. It leads to the induction and maintenance of a 'quiescent' phenotype in capillaries. Ang-1 also protects against airway inflammation and hyperreactivity in asthma [43,44], reduces thrombin-induced interleukin-8 production and neutrophil adhesion [45], decreases VEGF-induced up-regulation of inflammatory adhesion molecules [46], prevents endothelial hyperpermeability, thereby maintaining vessel integrity, and inhibits leukocyte-endothelium interactions [27,30]. Ang-1 and VEGF-A are thought to have a complementary effect on blood vessel growth which could be due to both ligands being able to activate the receptor Tie-2 (Fig. 1).

The release of Ang-1 protein from neutrophils differs from secretion of VEGF-A, because Ang-1 is cytosolic and its release is neither dependent on calcium, TNF-α nor other neutrophil-activating cytokines [47]. Ang-1 is expressed in the prostate, skeletal muscle, small intestine, heart, and areas of the brain [26].

Targeted disruption of Tie-2 or Ang-1 results in embryonic lethality, which occurs at a slightly later stage than that seen in VEGF-A knockout animals [48]. However, in the postnatal vasculature, Ang-1 appears to play a predominant role in the maintenance of endothelial homeostasis and prevention of vascular inflammation through the activation of Tie-2 [49].

Ang-1 and Ang-2 have been shown to interact with other receptors, including integrins [50]. Monomeric DeltaAng-1 has been observed binding $\alpha5\beta1$ integrin with similar affinity compared to Tie-2 (Fig. 1). This suggests that angiopoietins could be involved in mediating cell adhesion or migration through integrins independent of the Tie-2 receptor [51]. Moreover, in colonic samples from CD and UC patients, intestinal lamina propria mononuclear cells CD19+ B cells expressed more $\alpha5$ integrin than normal specimens [52].

2.5. Angiopoietin-2

Another major factor involved in angiogenesis, and discovered by sequence homology to Ang-1, was Ang-2 [26]. The functional role of Ang-2 is to act as a competitive antagonist of Ang-1 on ECs and VSMCs. It down-regulates Tie-2 signaling, thereby releasing the vascular endothelium from the strengthening inhibitory influence of Ang-1 [53].

Ang-2 facilitates EC activation in response to VEGF-A and other classic growth factors, and a given pathophysiological condition such as hypoxia [54]. It promotes angiogenesis by allowing ECs to be more responsive to VEGF-A mediated cell proliferation which is necessary for EC repair and turn over [38,39,55]. In the absence of VEGF-A, Ang-2 destabilizes the interaction between ECs and their support cells, causing plasma leakage and promoting vascular regression [26,56–60].

However, recent studies have demonstrated that the activity of Ang-2 is more complex than previously believed. When it binds Tie-2 without inducing phosphorylation, Ang-2 interrupts essential Ang-1/Tie-2 signaling, acting mainly as a functional antagonist of Ang-1 in an autocrine way [15,22,23,26,42,61]. Such beneficial or detrimental effects on inflammation depend not only on the phosphorylation of the Tie-2 receptor, but also on the duration of its particular receptor binding [37,38,41,62,63].

Ang-2 shows agonist activity binding Tie-2 in the absence of Ang-1; however, it functions as a dose-dependent antagonist when Ang-1 is present. ECs produce Ang-2 but not Ang-1, and

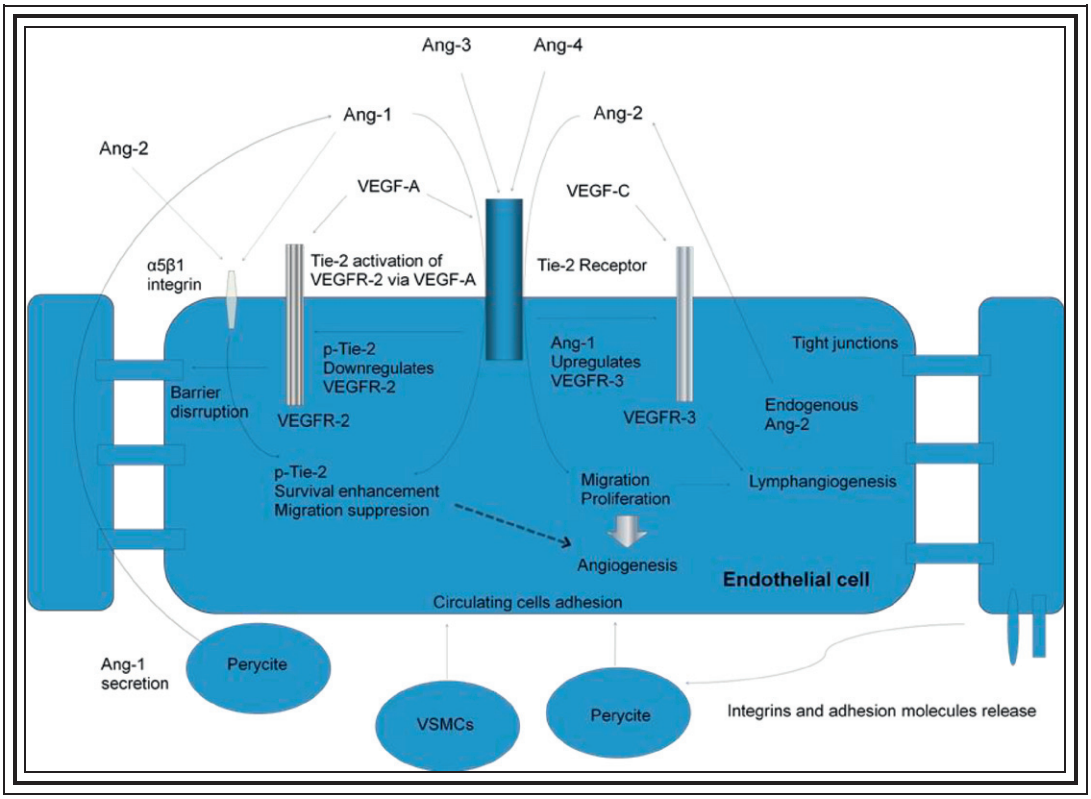

Figure 1 Schematic representation of possible Ang/Tie-2 signaling between vascular endothelial and smooth muscle cells and pericytes. Ang-1 multimers bind to Tie-2 on ECs, induces receptor phosphorylation and behave as an anti-inflammatory cytokine. Phosphorylated Tie-2 also down-regulates VEGFR-2 expression. When Ang-1 binds Tie-2 without phosphorylation, VEGFR-3 is up-regulated inducing lymphangiogenesis. Conversely, Ang-2 normally functions as an Ang-1 antagonist and mediates increases in vascular permeability and primes the vasculature for angiogenesis, but it could also function as a partial Tie-2 agonist under certain conditions: without inducing phosphorylation, Ang-2 interrupts Ang-1/Tie-2 signaling. In the absence of Ang-1, Ang-2 shows an agonist activity in a dose-dependent manner, and endogenous Ang-2 maintains Tie-2 activation. Both Ang-1 and Ang-2 can bind $\alpha5\beta1$ integrin similarly to Tie-2. Ang-3 and Ang-4 anchor Tie-2 as agonist and antagonist, respectively, but their exact function remains unclear.

this endogenous Ang-2 maintains EC survival, migration and tube formation. When these cells are stimulated with Ang-1 and -2, Ang-2 dose-dependently inhibits Ang-1-induced EC survival. Although Ang-2 is a weaker agonist than Ang-1, endogenous Ang-2 maintains a level of Tie-2 activation that is critical to a spectrum of EC functions (Fig. 1) [64,65]. This dose-dependent modulation of Ang-2 activity may be important in the regulation of neovessel formation because one ligand can mediate both the initial inhibition of Tie-2 activity necessary for an efficient early angiogenic response, as well as later maturation and stabilization of the neovasculature [28]. This view was challenged by the observation that high level expression of Ang-2 is present in both highly malignant tumors such as glioblastoma multiforme and inflammatory conditions such as psoriasis, IBD and hemangiomas [53,64,66,67].

Ang-2 activates multiple signaling pathways including reactive oxygen generation. It also plays a role by sensitizing endothelial cells to tumor necrosis factor (TNF-α) and can exaggerate inflammatory responses [37,68]. In adult human tissues, Ang-2 expression is largely restricted to sites of active vascular remodeling such as ovaries, uterus and placenta, while Ang-1 is more widespread [26].

2.6. Angiopoietin-3/Angiopoietin-4

Very little is known about these ligands. Ang-3 is thought to act as an agonist while Ang-4 exerts an antagonist effect at the Tie-2 receptor (Fig. 1). Their expression patterns also differ, as Ang-3 is expressed throughout the body and Ang-4 is detected only in lung [37,69]. Currently, the exact biological actions and pattern of Tie-2 signaling by Ang-3 and -4 are poorly characterized but their function seems to be cell specific [33,34].

2.7. Angiopoietins and the lymphatic system

Lymphatic vessels, which drain interstitial fluids, and return them back to circulation, are thought to grow following blood capillaries, in order to recover tissue homeostasis [70,71]. Ang-2 is known to be involved in the regulation of embryonic lymphangiogenesis. However, the role of Ang-2 in postnatal pathological lymphangiogenesis, such as inflammation, is largely unknown [72].

Although several lymphangiogenic factors are known to be involved in the formation of lymphatic vessels, the molecular

mechanisms that maintain lymphatic integrity and control the resolution of inflammation remain unclear. *In vitro* studies revealed that, during inflammation, the stability, integrity and function of lymphatic endothelial cells are enhanced in the presence of Ang-1 [73].

In addition to blood vessel organization, Ang-2 deficiency also influences the development of lymphatic vessels, particularly those within the gastrointestinal system [74]. In this respect, Ang-2 deficient mice have been reported as a relevant model of intestinal lymphatic dysplasia [75].

2.8. Angiopoietins in animal models

Recently, Cho et al. [76] designed an Ang-1 variant, COMP-Ang-1, which was more potent than native Ang-1 in phosphorylating the Tie-2 receptor in lung ECs *in vivo*. After its administration to mice, it was mainly located in microvascular ECs of the intestinal villi and lung. The Ang-1 variant prolonged survival in irradiated mice, showing specific protection against EC injury. There is evidence that Ang-2 deficient mice show complex defects in vascular development and postnatal vascular remodeling [29,77,78]. In IBD mouse models, blockade of Ang-2 with L1-10, an Ang-2-specific inhibitor, significantly inhibited lymphangiogenesis but promoted angiogenesis. These results clearly indicate that Ang-2 acts as a crucial regulator of inflammatory lymphangiogenesis by sensitizing the lymphatic vasculature to inflammatory stimuli, thereby directly promoting lymphangiogenesis [72].

In the development of experimental IBD in Ang-2(−/−) DSS treated mice, leukocyte infiltration, inflammation and blood and lymphatic vessel density have been shown to be significantly attenuated compared with wild type DSS treated mice. Ang-2 mediates inflammatory angiogenesis, lymphangiogenesis and neutrophil infiltration to reduce some, but not all clinical features of IBD.[5] However, in DSS induced endoglin heterozygous, Eng(+/−) mice model, Ang-1 and Ang-2 levels remained unchanged in the distal colon during DSS induction. In contrast, VEGF-A levels and vascular permeability were increased in the chronic phase of colitis [8].

2.9. Role of angiopoietins in IBD

Koutroubakis et al. first measured circulating serum levels of Ang-2 and Tie-2 in patients with IBD [79]. Both angiogenic factors were slightly elevated in patients with CD and UC compared with controls and Ang-2 also correlated with disease activity. Significantly higher levels of Ang-2 in patients with active IBD compared with patients with non-active disease were found. Both Ang-2 and Tie-2 median serum levels were significantly higher and lower, respectively, in those patients with early disease (diagnosis < 2 years) compared with IBD patients with late disease diagnosis [79]. A second study, where Ang-1 levels were also assessed, supported these observations in CD. In contrast to what resulted in Ang-2 and Tie-2, levels of Ang-1 were lower in patients with CD compared with healthy controls. This imbalance between Ang-1 and Ang-2 may reflect an abnormal process of maturation and stabilization of the vascular network during the course of IBD. Several correlations among angiopoietins and acute-phase reactants were also found [80].

A later report also suggested that, besides the potential role of angiopoietin circulating levels as disease markers, the Ang/Tie pathway may play a role in the local progression of UC by studying tissue samples from colonic mucosa [81]. The authors found that epithelia of crypt abscesses were strongly positive for Ang-1 and -2 in samples derived from patients with active UC, though the colorectal epithelium without crypt abscess showed minimal expression of Ang-1, Ang-2, and Tie-2. Specimens from UC patients in remission showed significantly less immunoreactivity for Ang-1, -2, or Tie-2. Therefore, they confirmed that the angiogenic response is induced by Ang-2, in the presence of VEGF-A, blocking the normal vessel-stabilizing effect of Ang-1 [81]. These results, the high immunohistochemical expression of Ang-1 in mucosal samples from patients with endoscopically-active UC compared with non-active UC patients, is similar to that of our group's current investigation. Moreover, we also found a high microvessel density in the same high Ang-1-expressing samples from active compared with non-active UC patients. Nevertheless, we have also included patients with CD, but no differences in Ang-1 expression and microvessel density were found when patients with active and inactive CD were compared [82].

These results concur with those of a large study performed in IBD patients in which CD patients with disease restricted to the colon had significantly lower serum Ang-2 levels in comparison with other phenotype locations, probably due to a transient variability or heterogeneity of endothelial cell characteristics [83,84]. Additionally, the authors found higher serum Ang-1 levels in UC patients who were smokers compared with non-smokers. This increase of serum Ang-1 in smoking UC, but not in CD, patients may be related to the nicotine effect on angiogenesis, which is known to up-regulate VEGF [85,86]. This probably suggests a collaboration between the VEGF and the angiopoietin system in UC which does not occur in CD [80]. On the other hand, no statistically significant difference in serum Ang-1 and -2 levels related to disease duration or activity was established, in contrast with previously reported data for serum Ang-2 [83,84].

Recently, Pousa et al. evaluated serum angiopoietin levels in patients with UC who underwent oral corticosteroid treatment. At baseline, Ang-1 levels were statistically significant lower than those in healthy individuals while Ang-2 levels, together with VEGF-A, were higher than in controls. In the same work, no correlation between angiopoietin levels and clinical activity of UC was found [87].

In this study, after treatment, patients who achieved complete remission showed statistically significantly higher serum levels of Ang-1 and Ang-2, but not of Tie-2, compared with those that did not achieve remission. Moreover, after completing treatment, Ang-1 and Ang-2 concentrations nearly returned to their baseline levels [87]. Therefore, corticosteroids temporarily alter circulating levels of VEGF and angiopoietins.

We are currently studying the effect of anti-TNF-α treatment on angiopoietin levels in patients with IBD. However, in contrast to the angiopoietin level modification seen during corticosteroid treatment, we have not observed changes in the levels of these factors after treatment. Although Ang-2 can mediate inflammation by up-regulating the response of endothelial cells to TNF-α, the effectiveness of anti-TNF-α treatment appears to not be related to the changes in the levels of these angiogenic factors [88].

In addition to the potential of the serum angiopoietin system levels as optimal markers of clinical activity, we also

evaluated histological and clinical activity. We studied the angiopoietin system in both serum and culture supernatant of mucosal samples [89,90]. We found that serum Ang-1 levels were higher in patients with endoscopically active IBD compared with non-active ones. In culture supernatant, levels of Ang-1, Ang-2 and Tie-2 were higher in patients with active compared with non-active IBD and Ang-2 levels of patients in remission were even lower than in controls. Probably, during periods of quiescence, lower levels of Ang-2 are released, as the Ang-2-expressing ECs recruitment ends, but the initial angiogenic balance is not totally restored. Also, there was a positive correlation between serum and colonic mucosa culture supernatant levels of Ang-1. Interestingly, we found differences of mucosal Tie-2 levels between affected and non-affected colonic area [89,90].

Ang-1 concentrations were modified in parallel with the severity of histological lesions, and moderately correlated with clinical and endoscopic activity. Thus, that the measurement of Ang-1 levels in serum may be useful as a non-invasive method should be put cautiously, as the area under the receiver operator Characteristic (ROC) curve for the diagnosis of disease activity for Ang-1 was below 0.7 for serum levels and 0.8 for mucosal culture supernatant [89,90]. Therefore, serum measurement of angiopoietins is not yet completely set up to avoid unnecessary colonoscopies.

3. Conclusions

The angiopoietin system is implicated in the regulation of the angiogenic process in sustained inflammation. These factors are also essential for vascular development, maturation and stability. As IBD is triggered by an activation of the immune system characterized by a chronic inflammation located in the gut, disease progression has been shown to be maintained by angiogenesis in a mutually dependent association. Nevertheless, the direct implication of the angiopoietin system in the development of intestinal inflammation is not fully understood. No less important is that until now, most of the studies are focused on the determination of these factors at a circulating level, which present high variability. Taking into account that cellular function and response depend on the surrounding environmental protein network, the exploration of changes in the distribution and association of angiopoietins with the mucosal activity that defines critical events involved in the disease's pathogenesis is required. Therefore, larger-scale studies in patients with IBD are needed to further determine the exact mechanisms that associate inflammation and angiopoietin levels.

Conflict of interest

There is no conflict of interest.

Acknowledgments

Pablo M Linares has performed and designed the research study and wrote the paper. María Chaparro and Javier P. Gisbert have contributed to the design of the study.

All authors approved the final version of the manuscript.

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