The potential role of angiogenesis in the development of shoulder pain, shoulder dysfunction, and lymphedema after breast cancer treatment

Abstract: Shoulder morbidity is a well-documented sequela of breast cancer treatment, which includes various manifestations such as pain, reduced range of motion, and lymphedema, among others. The multifactorial nature of such morbidities has long been appreciated, and research on reliable risk predictors of development thereof still continues. Previous studies have demonstrated the potential of different types of physical therapy to treat such shoulder problems, and the integration of such interventions into routine care for breast cancer survivors is a requirement in most high-income countries. Although patients at risk for developing shoulder problems would most likely benefit from posttreatment physical therapy, currently, there is no gold standard for identifying this patient group. This is particularly important in low- and middle-income countries where scarce monetary resources need to be directed specifically to those most in need. Modulators of the angiogenesis pathway have been implicated in noncancer shoulder conditions such as rotator cuff disease, adhesive capsulitis, and tendon injuries. The present review summarizes the role of angiogenesis in the development of shoulder morbidity among breast cancer survivors and sets forth the rationale for our belief that angiogenesis signaling may help explain a proportion of the reported clinical variability noted in the development of shoulder pain and dysfunction and upper-limb lymphedema after breast cancer treatment.

Keywords: angiogenesis, shoulder dysfunction, cytokines, polymorphism, breast cancer therapy

Introduction
Among women, breast cancer is both the most common cause of cancer-related deaths and the most frequently diagnosed type of cancer (~25% of all cancers) worldwide. The International Agency for Research on Cancer estimated 1.7 million new breast cancer cases among women worldwide in 2012, while in South Africa, an age-standardized incidence rate of 31.4 per 100,000 was estimated in 2011 as provided by the National Cancer Registry. More recent prevalence or incidence rates have not yet been established, either globally or in South Africa.

High 5-year survival rates of up to 89.2% have been reported following breast cancer treatment. Although generally lower in low-income countries, survival rates will most likely continue to rise due to advances in treatment, increased awareness, and early detection. Nevertheless, a significant proportion of breast cancer survivors experience upper extremity problems after treatment, which include pain, tightness, numbness, lymphedema, and limited range of motion, among others. Moreover, previous studies have suggested that shoulder morbidity after breast cancer treatment may
persist longer than is commonly reported.5,10,11 Although substantial variability exists among previous reports, 20%–32% of breast cancer survivors report arm lymphedema and pain or dysfunction in the shoulder, arm, or breast at least 1 year after primary treatment.5,12–17 These side effects, especially at the shoulder or arm, reduce the functional use of the upper limb of affected patients, limiting their quality of life and ability to return to work.5,18,19 The shoulder girdle comprises joints (glenohumeral, acromioclavicular, and sternoclavicular joints), associated muscles, and connective tissue connecting the upper arm and shoulder area, providing a mechanism for upper-limb motion. Breast cancer treatment is believed to place survivors at risk of developing impaired resting shoulder girdle alignment.20 The altered shoulder movement patterns observed in breast cancer survivors mimic those seen in known general shoulder conditions such as rotator cuff disease and adhesive capsulitis.21 In fact, such diagnoses have been used to describe shoulder and arm morbidities in breast cancer survivors and have been associated with pain.20,22,23 It is believed that breast cancer treatment-related impairments of the shoulder complex place breast cancer survivors at risk of developing symptomatic rotator cuff disease.20

There is substantial evidence supporting the effectiveness of different types of physical therapy in reducing upper-limb pain and lymphedema and improving shoulder range of motion (and, thus, shoulder function) in breast cancer survivors experiencing upper-limb morbidity.24–28 Nonetheless, the complex etiology of shoulder morbidity in breast cancer survivors has long been appreciated, with both treatment-related and patient-related factors implicated.10,12,15,17,20,29–37 These factors include treatment type (e.g., type of surgery and the extent of lymph node surgery), time after treatment, disease characteristics, age, genetic factors, and the presence of comorbidities and other phenotypes such as high BMI. However, a large proportion of the variation in developing pain, and perhaps other morbidities as well, following breast cancer treatment still remains unexplained.31,38 For instance, Wang et al reviewed that absolute risk increases of only 3%–21% in developing persistent pain following breast cancer treatment can be explained from a total of 77 factors.31 Previous findings suggest that shoulder morbidity after breast cancer treatment is bilateral,10,30,36 and it has been shown that “structures unrelated to direct surgery and/or radiotherapy treatment are affected,”39 suggesting a systemic cause. These collective findings suggest the need to focus on molecular signaling pathways.

Fibrogenic and inflammatory molecular signaling pathways have been implicated in the development of morbidity following breast cancer treatment as well as other forms of cancer.40–46 Although such signaling pathways constitute normal healing and repair processes, aberrant expression of signaling factors may underlie pathological processes such as persistent pain, swelling, and dysfunction of the shoulder complex reported among breast cancer survivors. According to the central dogma of molecular biology, it would follow that genetics may also be an important factor. Indeed, evidence suggesting specific single-nucleotide polymorphism (SNP)-modulated variation in inflammatory gene expression has been reported in studies on musculoskeletal pain following breast cancer treatment with aromatase inhibitors.47,48 Signaling pathways of inflammation, fibrosis, and angiogenesis have several links. In addition to fibrosis and inflammation, angiogenesis has been implicated in noncancer shoulder conditions such as rotator cuff disease49–51 and adhesive capsulitis.52 Angiogenesis has also been implicated in healing and adaptation pathways in noncancer musculoskeletal conditions such as tendon injuries53 and in the physiological response to mechanical loading.54,55 Evaluation of angiogenesis signaling factors in breast cancer survivors may, therefore, increase our understanding of the pathophysiology of upper-limb morbidities in this context and, perhaps, help explain a proportion of the variability in the development of such morbidities. In this review, we discuss the aspects of angiogenesis-related signaling factors as potential markers of risk for the development of shoulder morbidity after breast cancer treatment.

Ethical approval
The current review is part of an ongoing study approved by the Human Research Ethics Committee at the University of Cape Town, South Africa.

Literature search and selection
Database searches for articles and reviews were conducted in the following databases: PubMed, Scopus, Web of Science, MEDLINE, and CINAHL for articles published from January 1990 up to July 2016 using the following search terms: (“breast cancer” OR “breast carcinoma” OR “breast neoplasm*” OR “breast tumor”) AND (“treatment” OR “therapy”) AND (“shoulder pain” OR “shoulder dysfunction” OR “lymphoedema” OR “lymphedema” OR “range of motion”) AND (“angiogenesis” OR “inflammation”). A search was also conducted using the same terms excluding (“breast cancer” OR “breast carcinoma” OR “breast neoplasm*” OR “breast tumor”) and (“treatment” OR “therapy”) to capture articles implicating angiogenesis in general shoulder conditions independent of breast cancer treatment. The articles were
narrowed down by analyzing the titles and then the abstracts. We retained articles and reviews on the pathophysiology of shoulder pain and dysfunction both in breast cancer survivors and in noncancer patients. Trials on the efficacy of treatment of shoulder pathology were excluded.

Angiogenesis

Angiogenesis, the formation of new blood vessels from the endothelium of existing vasculature, is a fundamental process that occurs in both physiological and pathophysiological situations.\textsuperscript{56} It is a complex process that results in the transition of an endothelial cell (EC) from a quiescent to angio-active phenotype, through the activation of vital cellular events including EC activation, proliferation, migration, survival, and extracellular matrix remodeling\textsuperscript{57} (Figure 1). Angiogenesis regulation involves two main transcription factors, nuclear factor \(\kappa\)B (NF-\(\kappa\)B) and hypoxia-inducible factors (HIFs), in separate molecular pathways that induce expression of angiogenesis-associated signaling factors (cytokines, growth factors, and receptors).\textsuperscript{56} The transcription factor NF-\(\kappa\)B links angiogenesis and inflammation processes.\textsuperscript{56} Furthermore, inflammatory cells participate in the regulation of angiogenesis by secreting proinflammatory cytokines that directly or indirectly influence the activity of ECs.\textsuperscript{58}

Vascular endothelial growth factor-A (VEGF-A), the main angiogenesis signaling factor, stimulates EC proliferation, survival, and migration, mainly through its receptor VEGF-R2 (also known as KDR; Figure 1).\textsuperscript{56,57} Matrix metalloproteinases (MMPs) catalyze the proteolytic degradation of the basement membrane, which precedes EC migration, and their activity is regulated by tissue inhibitors of metalloproteinases (TIMPs).\textsuperscript{57} Thrombospondins (THBS-1 or -2) mediate antiangiogenic effects through the suppression of VEGF-A bioavailability and the inhibition of its activity through KDR.\textsuperscript{59} VEGF-A signaling through KDR upregulates some MMPs and endothelial nitric oxide synthase (eNOS), and the later catalyzes nitric oxide (NO) production which stimulates vascular permeability, an essential event in angiogenesis.\textsuperscript{60,61} The adhesion molecules vascular cell adhesion molecules (VCAMs) and intercellular adhesion molecules,
which mediate leukocyte infiltration, are also important components of angiogenesis.\textsuperscript{62,63} VEGFR-1 also known as FLT-1, with its relatively higher affinity for VEGF-A and weak tyrosine kinase activity, is believed to reduce VEGF-A bioavailability to the main angiogenesis signaling receptor, KDR, and is therefore antiangiogenic.\textsuperscript{57,64} Even though the links to angiogenesis are not included, for simplicity, it is noteworthy that the proinflammatory cytokines IL-1\textbeta, IL-6, and tumor necrosis factor-alpha (TNF-\textalpha) also possess proangiogenic effects and can transcriptionally be activated by HIF or NF-\kappaB\textsuperscript{56,58} (Figure 1).

The direct determination of angiogenesis involves investigation of changes in microvessel density, which may be done using ultrasound examination or immunohistochemistry of tissue biopsies. However, angiogenesis-related factors may provide useful information regarding angiogenic potential. The occurrence of angiogenesis depends on the balance between proangiogenic and antiangiogenic molecules in the tissue microenvironment\textsuperscript{65} (Table 1). Upregulation of proangiogenic factors promotes angiogenesis by facilitating the migration, proliferation, and differentiation of ECs into newly formed capillaries that can subsequently develop into more mature vessels.\textsuperscript{57,65} The HIF and NF-\kappaB signaling pathways crossregulate each other,\textsuperscript{56,57} and in effect, the HIF pathway can be induced in nonhypoxic conditions such as in response to reactive oxygen species (ROS) and NO.\textsuperscript{56} Angiogenesis is also regulated by microRNAs, which either upregulate or downregulate specific components of the angiogenesis pathway.\textsuperscript{56,66}

### Role of angiogenesis in response to breast cancer treatment

Angiogenesis is, at least to some extent, involved in the physiological response of the tumor microenvironment, or surrounding tissue, to breast cancer treatment, especially the common adjuvant treatments radiotherapy and chemotherapy. Moreover, an imbalance of or changes in inflammatory angiogenesis-related cytokines (ARCs) have been demonstrated in breast cancer patients after treatment.\textsuperscript{67,68} For example, De Sanctis et al reported significant increases in levels of interleukins (IL[s]) IL-1\textbeta, IL-2, and IL-6 and TNF-\textalpha 4 weeks after adjuvant radiation therapy, particularly in patients presenting with fatigue.\textsuperscript{67} Although some angiogenesis signaling factors are immobilized by various means such as binding to extracellular matrix components, some are soluble and thus capable of mediating systemic effects. This may particularly be important in the development of morbidity among breast cancer survivors since it has been shown that structures outside the direct treatment field are also affected.\textsuperscript{59}

### Radiotherapy and angiogenesis

Radiotherapy involves exposure of the tumor site to a specific dose of ionizing radiation, usually given in fractions. More than 50% of early stage breast cancer patients receive radiation therapy either alone or as an adjuvant treatment.\textsuperscript{59} Ionizing radiation damages DNA, which induces an intracellular response that leads to death by apoptosis in susceptible cells.\textsuperscript{70} Rapid proliferating cells, such as tumor cells, are particularly sensitive to ionizing radiation but DNA damage is also induced in normal tissue, including ECs, due to the indiscriminate nature of the treatment.\textsuperscript{41} Ionizing radiation also acts indirectly through the generation of ROS and reactive nitrogen species which, in addition to damaging DNA, inhibit cellular enzymes.\textsuperscript{70,71} Consequently, when the cell’s antioxidant and DNA repair capacities are overwhelmed, cell death by apoptosis occurs.\textsuperscript{70,71}

The DNA damage from irradiation elicits a biological response in the tumor microenvironment, which involves the release of chemokines, cytokines, and adhesion molecules leading to altered cell–cell interactions and the influx of inflammatory cells, particularly in ECs of tumor and/or normal tissue.\textsuperscript{41,72,73} These mediators are involved in various molecular pathways including angiogenesis, and they may participate in the development of normal tissue damage characteristic of either early (occurring in \textlessthan6 months after irradiation) or late (occurring in \textgreater6 months after irradiation) radiation toxicity effects.\textsuperscript{74,75} Radiation also induces soft tissue fibrosis, which can cause stiffness in the shoulder girdle leading to reduced range of motion.\textsuperscript{70,76} Fibrosis is also linked to angiogenesis through its main signaling factor, transforming growth factor-beta (TGF-\beta), which can influence angiogenesis.\textsuperscript{37}

One of the late toxicity effects of radiation therapy is damage to the vascular system in the form of lesions, which

### Table 1 Examples of proangiogenic and antiangiogenic factors

<table>
<thead>
<tr>
<th>Proangiogenic</th>
<th>Antiangiogenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-A</td>
<td>Thrombospondins (TSP-1 and TSP-2)</td>
</tr>
<tr>
<td>PDGF</td>
<td>Angiostatin</td>
</tr>
<tr>
<td>SDF</td>
<td>IL-12</td>
</tr>
<tr>
<td>bFGF</td>
<td>Angiopoietin 2</td>
</tr>
<tr>
<td>TNF-\alpha</td>
<td>Endostatin</td>
</tr>
<tr>
<td>IL-8</td>
<td>Interferon-\alpha, -\beta, and -\gamma</td>
</tr>
<tr>
<td>NRP-1</td>
<td>FLT-1</td>
</tr>
</tbody>
</table>

**Abbreviations:** bFGF, basic fibroblast growth factor; IL, interleukin; NRP-1, neuropilin-1; PDGF, platelet-derived growth factor; TNF-\alpha, tumor necrosis factor-alpha; VEGF-A, vascular endothelial growth factor-A.
Angiogenesis signaling in upper-limb morbidity

Potential role in pain, inflammation, and dysfunction

Angiogenesis-related signaling factors such as IL-1β, IL-6, TNF-α, and COX-2 are known to contribute to pain hypersensitivity by inducing the production of prostaglandins and other proalgesic agents, which activate nociceptors. Lymph node dissection can lead to lymphedema by compromising the lymphatic system’s ability to return interstitial fluid to main circulation, which is important in the resolution of inflammation. However, pro-inflammatory ARCs such as IL-1β, IL-6, and TNF-α have the potential to aggravate lymphedema by enhancing inflammation. Some angiogenesis-related factors...
are involved in extracellular matrix remodeling (Figure 1). Abnormal collagen and matrix production can potentially contribute to stiffness in the shoulder joint capsule, which can lead to reductions in the range of motion.

**Implication in noncancer general shoulder conditions**

Previous studies suggest the importance of angiogenesis in noncancer musculoskeletal conditions of the shoulder such as tendon injuries, adhesive capsulitis, and rotator cuff disease. For example, such studies have demonstrated altered vascularity or potential to heal as determined from fibroblast cellularity, vascularity, and the presence of a significant inflammatory component in rotator cuff tears. Moreover, previous studies have demonstrated altered expression of ARCs in such noncancer general musculoskeletal conditions of the shoulder. For example, altered levels of cytokines including IL-1β, IL-8, and VEGF have been shown in rotator cuff disease, whereas IL-1β, TNF-α, PDGF, and TGF-β were implicated in the pathogenesis of adhesive capsulitis.

**Genetic aspects**

Evidence of a potential genetic component in the development of posttreatment morbidity in breast cancer survivors has been explored and reported in several studies. Most of these studies involved associations between gene polymorphisms with clinical outcomes such as lymphedema, breast pain, fatigue, and anxiety, covering a total of 17 SNPs and 4 haplotypes from 14 genes (Table 2). However, replication of these findings has not been achieved, largely due to methodological issues among the studies which include, but are not limited to, variations in population structure and size, and heterogeneity in follow-up periods (or period after surgery) and outcome measures. Moreover, to date, these studies have largely focused on inflammatory gene polymorphisms, with one exception. Clearly, angiogenesis is unexplored even though evidence shows it is linked to inflammation. Associations were reported for both increased and decreased risks of morbidity (Table 2). For example, one study reported an association between persistent breast pain following breast cancer surgery and a polymorphism within IL-1 receptor type 2 (IL1-R2 rs1,16,74,595 T>C) and also highlighted a haplotype, implicating the 3′ untranslated region within IL-10. In another study, an association between a polymorphism in NFKB2 gene and the risk of developing lymphedema after breast cancer treatment was reported. With the exception of lymphedema, the clinical outcomes these studies focused on were not shoulder complex-specific outcomes such as

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP/haplotype</th>
<th>Association</th>
<th>Risk</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOXC2</td>
<td>haplotype A03</td>
<td>Lymphedema</td>
<td>↓</td>
<td>Miaskowski et al</td>
</tr>
<tr>
<td>IL1B</td>
<td>rs16,944</td>
<td>Fatigue</td>
<td>↑</td>
<td>Collado-Hidalgo et al</td>
</tr>
<tr>
<td>IL1R2</td>
<td>rs1,16,74,595</td>
<td>Severe persistent breast pain</td>
<td>↑</td>
<td>Stephens et al</td>
</tr>
<tr>
<td>IL6</td>
<td>rs22,27,284</td>
<td>Lymphedema</td>
<td>↓</td>
<td>Leung et al</td>
</tr>
<tr>
<td>IL10</td>
<td>rs15,18,111</td>
<td>Lymphedema</td>
<td>↑</td>
<td>Bower et al</td>
</tr>
<tr>
<td>LCP2</td>
<td>rs3,15,721</td>
<td>Lymphedema</td>
<td>↓</td>
<td>Stephens et al</td>
</tr>
<tr>
<td>NFKB2</td>
<td>rs10,56,890</td>
<td>Lymphedema</td>
<td>↑</td>
<td>Leung et al</td>
</tr>
<tr>
<td>NRP2</td>
<td>rs8,49,530</td>
<td>Lymphedema</td>
<td>↓</td>
<td>Miaskowski et al</td>
</tr>
<tr>
<td>SYK</td>
<td>rs1,58,689</td>
<td>Lymphedema</td>
<td>↑</td>
<td>Miaskowski et al</td>
</tr>
<tr>
<td>TCL1A*</td>
<td>rs71,58,782</td>
<td>Musculoskeletal adverse events</td>
<td>↑</td>
<td>Ingle et al</td>
</tr>
<tr>
<td></td>
<td>rs71,59,713</td>
<td>Musculoskeletal adverse events</td>
<td>↑</td>
<td>Ingle et al</td>
</tr>
<tr>
<td></td>
<td>rs23,69,049</td>
<td>Musculoskeletal adverse events</td>
<td>↑</td>
<td>Ingle et al</td>
</tr>
<tr>
<td></td>
<td>rs1,18,49,538</td>
<td>Musculoskeletal adverse events</td>
<td>↑</td>
<td>Ingle et al</td>
</tr>
<tr>
<td>TNFA</td>
<td>rs18,00,629</td>
<td>Fatigue</td>
<td>↑</td>
<td>Bower et al</td>
</tr>
<tr>
<td></td>
<td>rs17,99,964</td>
<td>Higher anxiety levels</td>
<td>↓</td>
<td>Miaskowski et al</td>
</tr>
<tr>
<td></td>
<td>rs30,93,662</td>
<td>Higher anxiety levels</td>
<td>↑</td>
<td>Miaskowski et al</td>
</tr>
<tr>
<td>VCAM1</td>
<td>rs31,76,861</td>
<td>Lymphedema</td>
<td>↓</td>
<td>Miaskowski et al</td>
</tr>
<tr>
<td>VEGFC</td>
<td>haplotype B03</td>
<td>Lymphedema</td>
<td>↑</td>
<td>Miaskowski et al</td>
</tr>
</tbody>
</table>

Notes: TCL1A* gene implied due to proximity to the SNPs (all SNPs were [926-7000 bp]) proximal to the TCL1A gene. ↑, increase; ↓, decrease. Abbreviations: NF-kB, nuclear factor-kB; SNP, single-nucleotide polymorphism; TCL1A, T-cell leukemia 1A; VCAM, vascular cell adhesion molecules.
movement-related pain or dysfunction, which are known to reduce quality of life.4,35,44,47,91–93 There are indications of a genetic component in the development of noncancer musculoskeletal shoulder conditions, evident from the predisposition of siblings to develop rotator cuff disease or rotator cuff tears.49,94,95 In support of this, associations between several polymorphisms and risk of developing rotator cuff disease have been reported.96 Furthermore, previous studies have reported associations between polymorphisms in various genes including genes encoding collagen and angiogenesis-related factors and risks of musculoskeletal injuries in sport.97–100 These factors are components of matrix remodeling, which is linked to angiogenesis.

**Conclusion and knowledge gaps**

Angiogenesis is potentially involved in the etiology of shoulder pain, lymphedema, and dysfunction and may help explain a proportion of the interindividual variability in the development of such morbidity among breast cancer survivors. It is involved in the response of the tissue microenvironment to adjuvant cancer therapies and has potential roles in pain, lymphedema, and dysfunction pathways. Furthermore, angiogenesis has been shown to play a role in noncancer shoulder conditions such as rotator cuff disease.86–88 However, there is a paucity of relevant studies investigating its role in morbidity after breast cancer treatment. Although several studies have explored expression profiles of cytokines and growth factors in breast cancer patients, most of these have focused on a few clinical symptoms such as cognitive impairments, fatigue, and breast pain or general pain.6,7,48,78,80,81,101,102 Clearly, most of these symptoms do not reside at the shoulder complex, the site most associated with limitations in daily activities. In addition, most focus has been on inflammatory cytokines with one exception.34 Similarly, with one exception,34 candidate genetic associations have largely focused on polymorphisms in inflammatory cytokine genes. Indeed, the role of angiogenesis signaling in the development of shoulder complex morbidity after breast cancer treatment is unexplored despite that it is linked to inflammation. Therefore, there is a need to explore and characterize the potential of angiogenesis-associated signaling factors in explaining interindividual variability in the development of posttreatment morbidity in breast cancer survivors. It is noteworthy that most of the studies that explored signaling factors in posttreatment morbidities,6,7,48,78,80,81,101,102 with one exception,101 involved a relatively short follow-up period (or period after surgery), up to 6 months after treatment, whereas current evidence suggests that shoulder/arm morbidity in breast cancer patients can persist for up to 6 years or more after surgery.10,11 Molecular signaling pathways or symptoms may evolve over time, and there is a need for longer follow-up periods (or period after surgery) to investigate this phenomenon.

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**Disclosure**

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**References**

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