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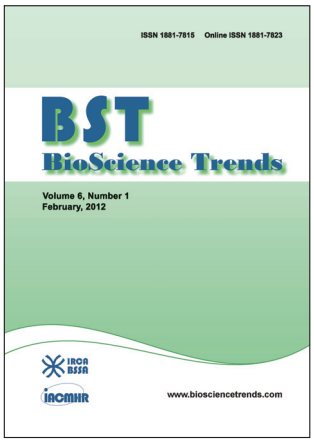

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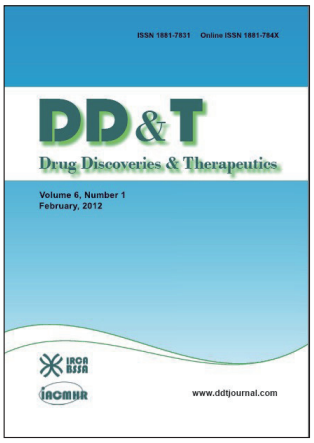

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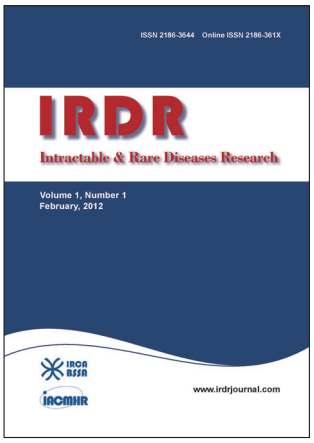

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Peripheral blood microRNAs: A novel tool for diagnosing disease?

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Summary **Peripheral blood microRNAs (miRNAs) are endogenous, noncoding small RNAs present in blood. Because of their size, abundance, tissue specificity, and relative stability in peripheral circulation, they offer great promise of becoming a novel noninvasive biomarker. However, the mechanism by which they are secreted, their biological function, and the reason for the existence of extracellular miRNAs are largely unclear. This article describes advances in the study of the mechanism of origin and biological function of extracellular miRNAs along with approaches adopted by research and questions that remain. This work also discusses the potential for peripheral blood miRNAs to serve as a diagnostic tool.**

Keywords: Peripheral blood miRNAs, diagnosis, biomarker, biological function

1. Introduction

The traditional method for diagnosing disease was to find pathological tissue. This method was highly specific but lacked sensitivity and involved greater harm to the patient. Therefore, the pressing need was to find a type of noninvasive and highly accurate method of diagnosing disease. MicroRNAs (miRNAs) are endogenous noncoding RNA molecules of 21-23 nucleotides that negatively regulate gene expression by binding to sites in the 3' untranslated regions of target mRNAs, causing a degradation or blockade of the translation. Since their discovery in the early 1990s, miRNAs have been found to play important regulatory roles in a wide range of biological and pathological processes. In recent years, rapid advances in sequencing techniques have greatly improved the sensitivity of their detection, and many miRNAs have been noted in serum and plasma. Studies have found that miRNAs are highly stable in peripheral blood containing ribozymes and some miRNAs, and their levels differ significantly in patients with different diseases (*1,2*). Furthermore, levels of expression of specific peripheral blood miRNAs are correlated with certain clinicopathological variables and could serve as a novel diagnostic biomarker for detection of disease and could be used clinically to monitor disease progression (*3-8*). However, some researchers began to question their diagnostic value given that peripheral blood miRNAs have not been found to play a functional role in the etiology or progression of disease (*9*). There are doubts about whether levels of expression of peripheral blood miRNAs can be regarded as diagnostic targets and the diagnostic value they may have.

2. miRNAs in peripheral blood

As an existing form of miRNAs, peripheral blood miRNAs come directly from exosomes, which are vesicles 30-100 nm in diameter. Since Johnstone *et al*. discovered that exosomes perform a number of activities (*10*), as exemplified by the reticulocyte plasma membrane in sheep reticulocytes cultured *in vitro*, exosomes have been gradually emerged from the shadows. Researchers found that purified exosomes contain functional miRNAs and originate from endocytic compartments that are released by many cell types (*11,12*). A study concerning the mechanism of exosome secretion found that miRNAs increased through overexpression of neutral sphingomyelinase 2 (nSMase2) (*13*). Decreasing the activity of nSMase2 with a chemical inhibitor, GW4869,

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and specific small interfering RNA resulted in the reduced secretion of miRNAs, so exosomes are released *via* ceramide-dependent secretion. Moreover, a study examining mammary epithelial cells releasing miRNAs revealed that mammary epithelial cells typically release similar exosomes (*14*). That study measured the level of expression of CD63 and CD81, endosomal marker proteins, and the authors considered extracellular miR-16 to be a surrogate marker for the abundance of endosomal miRNAs.

In addition, levels of miRNAs and exosomes in peripheral circulation are correlated with breast cancer (*5*), osteoarthritis (*15*), lung cancer (*16,17*), and ovarian cancer (*18*). The relative expression of some miRNAs is closely associated with clinicopathological features of cancer, such as their histologic grade and pathology. In short, these studies have highlighted the potential for use of exosomal miRNAs profiles as diagnostic biomarkers of disease through noninvasive testing. Nonetheless, cells selectively release miRNAs. Pigati *et al*. found that the bulk of miR-451 and miR-1246 produced by malignant mammary epithelial cells was released but that most of the miRNAs produced by non-malignant mammary epithelial cells were retained (*14*). In addition, Tanaka *et al*. found a high level of miR-92a expression in tissue samples from patients with acute leukemia but a reduced level of miR-92a in plasma (*19*). Lodes *et al*. also found that the expressional profiles of serum miRNAs did not directly correspond to tissue profiles (*20*). Therefore, some types of cells selectively release miRNAs, and other pathways of miRNAs secretion must exist.

A study of the secretary pathway of let-7, a tumor-suppressive miRNAs, that targeted oncogenes such as *RAS* and *HMGA2* found that not all types of cells secreted exosomes (*21*). Moreover, another study did not detect exosomal miRNAs in serum from normal controls (*20*). However, a variety of tissue-derived miRNAs exist in the peripheral circulation of healthy people (*22*). So where do these miRNAs come from? Some researchers believe that cell death or cell injury is the mechanism of miRNAs release (*7,23,24*). Alternatively, the release of miRNAs in exosomes is correlated with house-cleaning, whereby cells release damaged components and other cellular components into the environment (*25*). Regardless, none of the hypotheses can sufficiently explain the mechanism of miRNAs release. Therefore, the mechanisms involved in miRNAs release and whether different cells have the same mechanism are questions that still need to be determined.

3. Functions of peripheral blood miRNAs

As an important regulator in the post-transcriptional control of gene expression, miRNAs are involved in major biological processes of cancers, including metastasis, differentiation, apoptosis, and proliferation. In the blood, cellular interactions between erythrocytes, leukocytes, platelets, and endothelial cells are regulated by complex mechanisms that involve multiple molecules. Interestingly, exosome-derived miRNAs are one such molecule. Circulating miRNAs exist in the form of exosomes (*26*), which play an important role in intercellular communication, and mature miRNAs can be transferred between circulating cells through exosomes (*12*).

Many recent studies have noted that miRNAs are important participants in erythropoiesis (*27-30*), lymphopoiesis (*31-33*), the modulation of innate immune response (*34-36*), adaptive immune response (*37-39*), and the differentiation of leukocytes (*40*) and dendritic cells (DCs) (*41,42*). These findings show that exosomal miRNAs are crucial to the functioning of blood cells, and miRNAs could be manufactured into drugs to correct for autoimmune diseases or other diseases related to blood cells.

As miRNAs transporters, exosomes contain inactive miRNAs. Instead of a single messenger, exosomes can deliver multiple miRNAs at one time to neighboring cells and simultaneously suppress related genes (*43*). Thus, exosomes are a potential way to treat complex and uncontrollable diseases. However, the mechanism by which exosomes bind to the cellular surface and exosomal miRNAs enter into cells is not known. Also unknown is whether exosomes selectively bind to neighboring cells. Other pathways through which miRNAs enter cells need to be determined.

4. The potential use of peripheral blood miRNAs as a diagnostic tool

To deduce miRNAs involved in the progression of non-small-cell lung cancer (NSCLC), one study examined exosomes, another examined serum, and the third examined plasma (*16,44,45*). The three studies came up with different results. Other studies found that different techniques lead to different results (*46,47*). This suggests that appropriate specimens and techniques must be used for results to be generalizable and valid.

Whole blood, serum, and plasma can be used to study peripheral blood. Given its relation to erythrocytes, leukocytes, and platelets, whole blood is often chosen when studying immune diseases, inflammatory diseases, or coagulation factor deficiencies, while other studies choose serum or plasma. In actuality, the best specimens are ones that characterize the status of disease; in most diseases, exosomes are the best choice because their origin is known.

The qualitative and quantitative analysis of miRNAs is essential. Microarrays are often used to that end, as are quantitative real-time polymerase chain reaction (qRT-PCR) systems. Real-time PCR using the TaqMan Array Human MicroRNA panel is a novel and practical means of high-throughput investigation of serum RNA samples (*48*). Lodes *et al*. invented a type of microarray platform

that enables the simultaneous analysis of all human microRNAs *via* either fluorescent or electrochemical signals (*20*). This platform could easily be redesigned to include newly identified miRNAs without the need for amplification. Further developments have taken place. For example, Lusi *et al*. manufactured an electrochemical genosensor that is able to directly detect miRNAs without the need for PCR and a labeling reaction; their technique is simple, fast, and ultrasensitive (*49*). Heneghan *et al*. developed a reverse-transcription qRT-PCR assay that can detect circulating miRNAs in serum without RNA isolation (*9*). To improve accuracy, Moltzahn *et al*. invented a multiplex qRT-PCR technique involving purification of multiplex PCR products followed by uniplex analysis on a microfluidic chip to evaluate 384 human miRNAs (*3*).

5. Conclusion

Systems biology is defined as a comprehensive quantitative analysis of the manner in which all of the components of a biology system interact functionally over time (*50*). At the molecular level, the focus of systems biology is to determine the functioning of key molecules in cell signal transduction and gene regulation networks. miRNAs are one class of small, non-coding regulatory molecules, and they play an important role in diverse biological processes such as development, cell proliferation and differentiation, apoptosis, oncogenesis, metabolism, angiogenesis, and inflammation. Therefore, studying the functions of miRNAs is essential to understanding the mechanism of disease and to perceiving the internal workings of biosystems.

As an existing form of miRNAs, peripheral blood miRNAs are encased in exosomes where they are protected from enzymatic degradation. They bind to neighboring cells to regulate the expression of target genes. Most recent studies on peripheral blood miRNAs focused on whether peripheral blood miRNAs can serve as a novel noninvasive biomarker. Surprisingly, almost all found that some (tissue-specific or tissue-nonspecific) circulating miRNAs were correlated with the development and progression of disease. However, the origin and functioning of peripheral blood miRNAs are unclear. miRNAs may exist in another form when they act in peripheral blood.

Peripheral blood miRNAs offer promise in the area of prenatal diagnosis. Evidence has revealed that some placental-specific miRNAs are consistently detected in maternal serum or plasma (*2,51*). Further research demonstrated that chorionic villous trophoblasts continuously released placenta-specific miRNAs into maternal circulation *via* exosomes (*52*). The level of expression of placenta-specific miRNAs in maternal peripheral blood is closely related to pre-eclampsia (PE) (*3*), congenital heart defects (CHD) (*53*), and fetal growth restriction (*54*).

In addition, some specific miRNAs have better sensitivity and specificity when distinguishing healthy specimens from those with disease, such as the most common forms of cancers: breast (*6,7,55*), prostate (*47*), lung (*16,44*), and colorectal cancer (*56,57*). A study of the potential for miRNAs to serve as a biomarker in drug-induced liver injury found that specific miRNAs species exhibited dose- and exposure duration-dependent changes in the plasma that parallel the histopathology of liver degeneration and levels of serum aminotransferase, an earlier biomarker for liver injury, but their changes can be detected significantly earlier (*22*).

In conclusion, recent studies have shown that tissue-specific miRNAs in peripheral blood may be a potential biomarker because they are noninvasive and reproducible and also because they are accurate (sensitive and specific) and predictable. Therefore, peripheral blood miRNAs may offer a better tool for the diagnosis of disease, though some issues remain.

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Review

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Dry age-related macular degeneration: A currently unmet clinical need

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Summary **Age-related macular degeneration (AMD) is a leading cause of severe visual impairment and disability in older people worldwide. Although considerable advances in the management of the neovascular form of AMD have been made in the last decade, no therapy is yet available for the advanced dry form of AMD (geographic atrophy). This review focuses on current trends in the development of new therapies targeting specific pathophysiological pathways of dry AMD. Increased understanding of the complex mechanisms that underlie dry AMD will help to address this largely unmet clinical need.**

Keywords: Age-related macular degeneration, dry age-related macular, degeneration, geographic atrophy, treatment, clinical trials

1. Introduction

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness and visual disability in the elderly. AMD prevalence substantially increases with increasing age and up to one third of individuals aged 75 and older suffer from a form of AMD (*1*). According to the European Eye Study (EUREYE), an estimated 2.5 million of the European population 65 years and older have AMD and more than 1.1 million have significantly impaired vision due to bilateral AMD (*2*). A meta-analysis of population-based data estimated that AMD affects 1.75 million individuals aged 40 years and older in the United States, and owing to the rapidly aging population, this figure is projected to rise to almost 3 million by 2020 (*3*). Although some early data in the past suggested that AMD in non-Caucasian populations may be less common, recent population-based studies indicate that an increasing prevalence of AMD may be occurring elsewhere (*4-6*).

AMD is a gradually progressive disease that evolves through stages into severe central vision loss (Figures 1A and 1B). The earliest signs of AMD designated as age-related maculopathy (ARM) involve the appearance of extracellular deposits (drusen), subretinal deposits of oxidized lipids and proteins beneath the retinal pigment epithelium (RPE), and variable amounts of visible clumps of pigment in the macula. In the intermediate stages of AMD, drusen become larger and pigmentary changes are more severe. In the advanced stages, patients develop either subretinal choroidal neovascularization (the exudative or "wet" form of AMD) or the non-neovascular "dry" form of AMD. The dry form of AMD is characterized by sharply demarcated uni- or multi-focal regions of dysfunctional macula, termed geographic atrophy (GA) (Figure 2). The GA patches gradually enlarge to involve the RPE and the corresponding neurosensory retina and choriocapillaris layer of the choroid. These progressive and irreversible changes ultimately cause permanent loss of central (macular) vision. Areas of GA therefore correspond to absolute scotomas. GA occurs bilaterally

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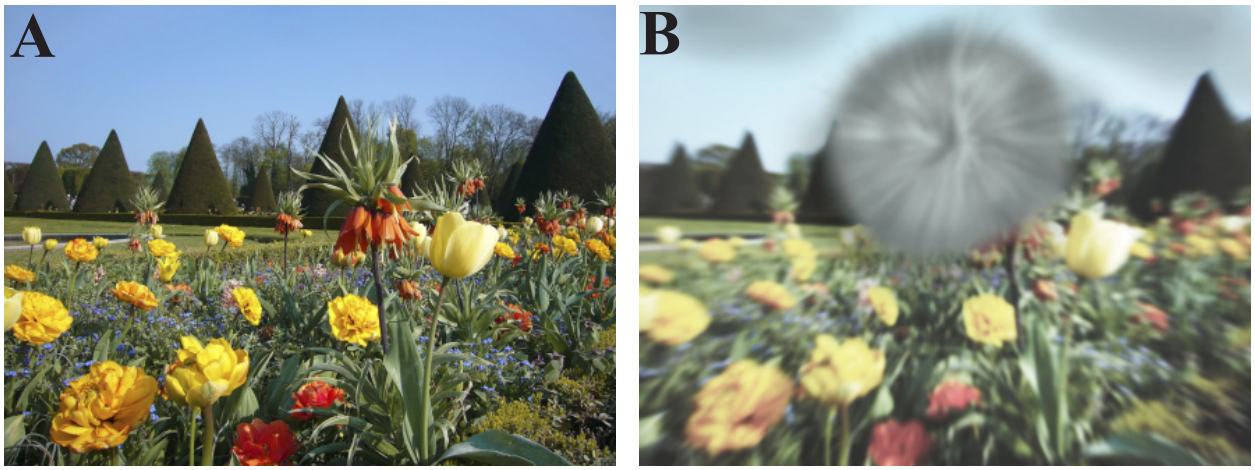

Figure 1. A scene viewed by a person with normal vision and age-related macular degeneration (AMD). (A) A scene as it might be viewed by a person with normal vision; **(B)** The same scene viewed by a person with age-related macular degeneration. (Image courtesy of Dr. Frangov)

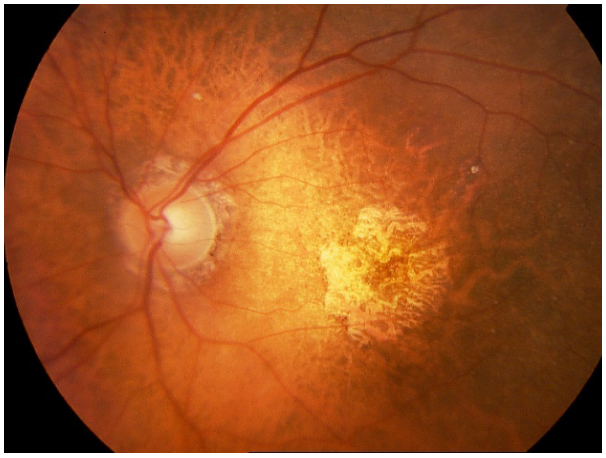

Figure 2. Fundus photograph showing geographic atrophy due to age-related macular degeneration. (Image courtesy of Dr. J-F Girmens)

in over 50% of patients, as reviewed (*7*). It represents the slower and more insidious form of the disease, and accounts for approximately 20-25% of patients with severe visual loss secondary to AMD (legal blindness related to AMD) (*8*) and for a much larger percentage of moderate visual loss of the elderly (*9*).

In recent years, significant progress has been made towards developing treatments for the wet form of AMD. Some of these treatment options demonstrated clear therapeutic success, including laser photocoagulation, photodynamic therapy, and most recently, intraocular drug therapy with anti-VEGF agents, such as ranibizumab (Lucentis®) or pegaptanib sodium (Macugen®). However, development of treatments for advanced dry AMD has not yet progressed either as quickly or to an extent comparable to those for the wet form of AMD. So far, no treatments have been proven to effectively prevent the onset of GA (*10*) or to halt lesion enlargement and/or retard vision loss (*11,12*). Taking into consideration the rapidly aging population throughout the world, the morbidity resulting from AMD becomes increasingly significant and dry AMD remains a large unmet clinical need. Its prevention would be of paramount socio-economic importance for the individual patients and for the healthcare system. Improved understanding of the pathogenesis of dry AMD would certainly provide a powerful basis for development of novel therapeutic strategies.

2. Pathophysiology and risk factors

AMD is a multifactorial disease with largely unknown pathophysiology. It is believed to be caused by cumulative damage over a lifetime leading to progressive deterioration of the RPE, Bruch's membrane and choriocapillaris-choroid complex (key players in maintaining retinal function), followed by photoreceptor cells damage. However, the locus of primary damage still remains unclear. Identified pathogenic mechanisms to date include a range of genetic and environmental factors related to primary RPE senescence, oxidative stress, alterations in the complement pathway, increased inflammation, changes in the balance of growth factors, and excessive lipofuscin accumulation (*1,13*).

Drusen, deposits of extracellular material beneath the RPE and within Bruch's membrane, are considered the hallmark of the disease. Factors that were correlated with increased risk of progression to advanced AMD include large initial soft drusen and large numbers of small hard drusen (*14*). As supported by epidemiological data, aging is considered to play a major role in AMD development. Only 2% of 50-60 year-old individuals have AMD, while the disease prevalence rises exponentially with increasing age so that over 30% of individuals over age 75 suffer some form of AMD (*15*). A key component of drusen is amyloid beta, a waste product which accumulates in the central nervous system with aging and in several age-related diseases, such as Alzheimer's disease. Age-

related accumulation of amyloid beta is documented along Bruch's membrane and blood vessels, and also in the photoreceptor outer segment (*16*). In AMD and aging, an increased oxidative stress induced by various intracellular photochemical reactions or normal metabolism is present in the retinal and choroidal tissue. Oxidative stress can result in blood-retinal barrier breakdown allowing plasma proteins and platelets to invade retinal tissue. Oxidative stress itself may contribute to further inflammatory responses, including complement activation and pro-inflammatory cytokine production. A potential role for lipofuscin in drusen biogenesis has also been proposed, even though the reasons for lipofuscin accumulation remain insufficiently understood.

Recently, inflammatory etiology of AMD (inflammation, immune system and autophagy) has been increasingly recognized and strengthened with the identification of complement factor H mutations being associated with over 40% of AMD cases (*17,18*). Complement is constitutively present in normal retina (and active at low levels) where it plays a vital defensive role against pathogens and helps to maintain immune privilege. In case of excessive activation, however, it may have a potentially harmful action. Since drusen include elements of the complement system belonging to all pathways, such as C1q, mannose binding lectin, factor B (CFB), factor I (CFI), factor H (CFH), C3 and its fragments − C5, many studies support the idea that the complement cascade plays an important role in the pathogeneses of AMD. It may act both as a trigger and progression factor, alone or together with other pathogenetic mechanisms, such as oxidative stress, although the molecular basis of these interactions remains to be determined. Other potential mediators of inflammation in AMD are the pro-inflammatory cytokines IL-1, IL-6, and TNFα, which are released from the choroid of patients with AMD.

In recent years, the whole genome screening allowed identification of mutations and polymorphisms in protein sequences of key regulators of the complement system associated with AMD. Thanks to this technological progress, heredity is now recognized to play an important role as an AMD risk factor, with major risk loci on chromosome 1 in the complement regulatory genes (*17*) and on chromosome 10 in the promoter region of the *HTRA1* gene (*19*). Genetic studies reported multiple polymorphisms in complement genes (including *CFH*, complement factor H-related (CFHR) 1 and 3, *CFB*, *C2*, *C3*, and *CFI*) as risk factors for AMD (*20*). Three initial studies have found an association between AMD and polymorphism of the *CFH* gene (Y402H) (*18,21,22*). Another member of the complement system, factor B, has also been shown to have both high-risk and protective variants associated with the development of AMD (*23*). A hypothetical gene *LOC387715*, residing in chromosome 10 in a region associated with susceptibility to AMD (subsequently reinstated with the symbol ARMS2), has been demonstrated to have an independent effect on AMD that is almost as strong as that of the *CFH* gene (*24,25*). A recent study (*26*) discovered that genetic variants near TIMP3 (a metalloproteinase involved in degradation of the extracellular matrix, previously implicated in early-onset maculopathy) and high-density lipoprotein (HDL)-associated loci (human hepatic lipase, LIPC, and cholesterol ester transfer protein, CETP) modify susceptibility to AMD. Consistent with the hypothesis that the HDL pathway is associated with AMD pathogenesis, these investigators identified two additional genes, lipoprotein lipase (*LPL*) and ATP-binding cassette transporter 1 (*ABCA1*). The genetic variants found in the cholesterol pathway are believed to impact the retina and may be a target for a future AMD therapeutic strategy. CFH and HTRA1 variants appear to predispose to both atrophic and neovascular AMD (*27,28*); however, the mechanisms that are specific to GA are still largely unknown. Recently, toll-like receptor-3 (TLR3) activation (enhanced with the 412L variant) has been proposed to specifically promote progression of the disease to the GA phenotype (*29*), but this association still remains controversial and needs further elucidation. Also, bone morphogenetic protein-4 (BMP4) has been found prominently and specifically expressed only in the RPE and adjacent extracellular matrix of patients with dry AMD, while almost no expression was observed in the same tissues of patients with wet AMD (*30*). These findings opened a new hope towards novel potential therapeutic solutions specifically targeting the dry form of AMD.

Environmental and demographic factors seem to be involved in the pathogenesis of AMD. Large population-based studies indicated that cigarette smoking and in particular, prior and current smokers are at increased risk for developing AMD, female smokers being at higher risk for progression to advanced AMD, while male smokers may be at higher risk for dry AMD (*31,32*). Obese individuals appear at higher risk for dry and neovascular AMD, while very lean individuals may be at higher risk for dry AMD (*33*). Alcohol consumption was not found to be associated with AMD, as reviewed (*34*). AMD appears to be more prevalent in the white population (*35*). Among demographic factors, the female sex, light skin pigmentation and light iris pigmentation may represent risk factors for AMD. Among environmental factors, excessive visible light exposure may be linked to the risk of developing AMD. Improving knowledge of the multiple genetic, environmental and demographic factors involved in the pathogenesis of the dry AMD is expected to enable development of targeted drugs to specifically treat and possibly even prevent the disease.

The contribution of epigenetic factors in the pathophysiology of AMD remains largely unknown. Recent investigations point at the unknown portion of genetic risk in AMD and the relevance of epigenetics in this respect (*36,37*). Genome-wide association studies would certainly help researchers enhance the global knowledge on the genetics of complex diseases such as AMD and its potential to propose targeted disease prevention or treatment. Future research is required to obtain valuable information on epigenetic regulation of AMD.

3. Current therapeutic strategies

No therapy is currently available for the dry slowly progressing atrophic form of AMD. Treatment directed against the cause of the disease yet remains difficult to accomplish, as the underlying etiology is very complex and remains elusive. This review focuses on recent trends in the development of new therapies targeting specific pathophysiological mechanisms of dry AMD. Special attention was paid to advanced treatments under evaluation in ongoing clinical investigations. The information on registered clinical trials and observational studies cited in this paper was obtained from the ClinicalTrials.gov Results Database assessed in May 2012.

3.1. *Supportive measures*

Without proven treatment to stop or prevent progression of the disease, patients should be offered supportive measures and appropriate advice on adequate lighting for near-vision tasks. Common low vision aids include low vision filters (including red or amber filters to reduce the severe photophobia, colored translucent acetate sheets to enhance contrast between the print and background), stand-mounted or handheld video magnifiers (*e.g.* closed circuit video magnification systems), electronic aids (*e.g.* autofocus systems, electronic visual enhancement systems, prismatic eye glasses to refract images from a field outside the normal central field), and low vision rehabilitation (*e.g.* eccentric fixation technique, which teaches patient to look just to one side of the object of interest rather than directly at the object). A newer approach to visual rehabilitation includes the implantable miniature telescope, a monocular-fixed focus telescopic device. It is implanted into the patient eye during surgery (*e.g.* cataract surgery) and projects images over healthy areas of the retina; after surgery, a vision rehabilitation program should be proposed (*38*). Excessive visible light exposure should be avoided and sunglasses should be advised (absorptive sunlenses/sunglasses). Cessation of smoking should be vigorously advised as it believed that this measure may reduce the risk of AMD progression.

3.2. *Drugs to prevent injury from micronutrient depletion and oxidative stress* (*micronutrient supplements and antioxidants*)

A variety of nutrients has been epidemiologically linked with decreased risk of AMD, including the antioxidant vitamins C, E, and A, and the zinc and selenium minerals that may act as co-factors for a number of endogenous antioxidant enzymes. Patients should be advised of the importance of a well-balanced diet including omega-3 fatty acids, which can be obtained from fish (salmon, tuna, mackerel, herring, and sardines), vegetable oils (soybean, rapeseed, linseed, and walnuts) or some green vegetables (Brussels sprouts, kale, spinach, and salad greens). Several epidemiological studies have indicated that diets rich in these particular lipids are associated with decreased risk of AMD. The Age-Related Eye Disease Study (AREDS) is the major clinical trial that was designed to obtain knowledge about the natural history and risk factors of AMD. This multi-center, controlled randomized study followed 4757 subjects, 55-80 years old, for at least five years. AREDS formulation included 500 mg of vitamin C, 400 IU of vitamin E, 15 mg of beta-carotene (often labeled as equivalent to 25,000 IU of vitamin A), 80 mg of zinc as zinc oxide and 2 mg of copper as cupric oxide (these high doses of zinc and anti-oxidants cannot be achieved from diet alone). Participants received daily oral tablets of either: 1) zinc alone; 2) antioxidants alone; 3) a combination of antioxidants and zinc; or 4) a placebo (*39-41*). The results of this study showed that a high-dose combination of vitamins and zinc significantly reduced (by about 25%) the risk of advanced AMD and associated vision loss in the high risk group of people with intermediate or advanced (late) AMD in one eye. There was no benefit in patients with no signs of AMD or early AMD, or those with bilateral advanced AMD. A few adverse effects have been registered in this study, *e.g.* yellowing of the skin and genitourinary complications (mostly due to antioxidant and zinc ingestion, respectively). Other studies, however, demonstrated that the beta-carotene component increases the risk of lung cancer in smokers, and this treatment should not be recommended in smokers or ex-smokers (*42*). The reduction of AMD progression risk upon using the AREDS therapeutic regimen can be considered as modest, but in view of the rapidly increasing aging of the population, the public health implications are enormous. This is why the recommendation of AREDS compliant formulas to at risk patients are considered as standard care in ophthalmology (*15*).

Among the large number of antioxidants, the dietary xanthophyll carotenoids lutein and zeaxanthin are of particular interest because they are specifically concentrated in the human macula (*15*). The first epidemiological evidence that lutein and zeaxanthin

may be protective against AMD was published in the 1990s by the Eye Disease Case-Control (EDCC) Study Group (*43*). The initial findings of this study demonstrated in 421 cases and 615 controls that there was an inverse correlation between serum carotenoid levels and risk of exudative AMD. In a follow-up study of a subset of these same patients, it was found that dietary consumption of fruits and vegetables rich in lutein and zeaxanthin was associated with a 43% decrease in risk of AMD, while diets rich in beta-carotene (which is not found in the retina and which cannot be converted to xanthophylls) were not protective (*43*).

The Lutein Antioxidant Supplementation Trial (LAST) reported that purified lutein (a naturally occurring molecule found in dark green leafy vegetables such as spinach, kale and collard greens) or a supplement mix of lutein and other antioxidants such as vitamin A, vitamin C, vitamin E, and beta carotene led to significant improvements in several objective measurements of visual function including glare recovery, contrast sensitivity, and visual acuity *vs.* placebo (*44*). The LAST trial studied 90 AMD patients supplemented daily with an OcuPower supplement capsule containing 10 mg of crystalline FloraGLO lutein (the average daily ingest is ~1-2 mg of lutein), 10 mg lutein plus a mixed antioxidant formula, or placebo for 12 months.

The AREDS 2 study (NCT00345176, Phase 3 clinical study, currently ongoing) was initiated in 2006 to study the effects of two dietary xanthophylls (lutein and zeaxanthin) and two omega-3 long-chain polyunsaturated fatty acids (LCPUFAs) – docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), on progression to advanced AMD and/or moderate vision loss. AREDS 2 is similar in scale to the original AREDS study with 4,000 subjects (age 50-85 with a high risk for AMD progression: large drusen in both eyes or advanced AMD in one eye and large drusen or non-central geographic atrophy in the fellow eye) who will be followed for five years at 100 sites. AREDS 2 formulation includes lutein 10 mg/ zeaxanthin 2 mg, LCPUFA ~ 1 g (350 mg DHA, 650 mg EPA). These micronutrients are believed to function not only as antioxidants, but also as anti-inflammatory and antiangiogenic agents.

The etiologic relevance of oxidative stress indicates a possible benefit in reducing free radicals in the AMD retina. OT-551 (Othera Pharmaceuticals) is one of the agents with antioxidant properties that are under investigation in dry AMD. It is a small lipophilic molecule, a disubstituted hydroxylamine, that readily penetrates the cornea. Converted by ocular esterases, the active metabolite has potent free-radical scavenger and antioxidant activities and, possibly, anti-inflammatory and antiangiogenic effects. OT-551 was studied in a single-center, open-label phase 2 trial, enrolling 10 participants with bilateral GA (topical 0.45% application, three times daily for up to three years) (*45*). Although well tolerated, OT-551 had no significant effect on lesion enlargement, retinal sensitivity or total drusen area compared to control fellow eye; a possible effect in maintaining visual acuity was suggested. It was concluded that the current concentration and mode of delivery have limited or no benefit as a treatment for GA (NCT00306488, completed).

3.3. *Drugs to preserve photoreceptors and RPE through neuroprotection, improved blood supply and metabolic modulation*

Ciliary neurotrophic factor (CNTF) is a cytokine member of the IL-6 family and a potent neuroprotective agent shown to rescue photoreceptors from degeneration in a number of preclinical studies, as reviewed (*46*). Intravitreal encapsulated CNTF sustained-release platform (CNTF/NT-501) that produces CNTF for a year or longer was developed by Neurotech Pharmaceuticals. It is known as an encapsulated cell technology (ECT) implant. A Phase 1 trial carried out in ten patients with advanced retinitis pigmentosa for a period of six months demonstrated that CNTF delivered by ECT implant could safely be implanted leading to subjective and objective visual acuity improvement (*47,48*). A randomized Phase 2 trial of a NT-501 implant for patients with atrophic macular degeneration was recently completed (NCT00447954). It showed that both the implant and the implant procedure were well-tolerated. CNTF treatment resulted in a dose-dependent increase in retinal thickness and apparent stabilization of visual acuity (*49*). These investigators concluded that the CNTF-ECT implant appears to slow the progression of vision loss in GA, especially in eyes with 20/63 or better vision at baseline. Further studies are warranted to establish this benefit.

Brimonidine (Allergan Inc., Irvine, CA, USA) is believed to have neuroprotective properties (based on animal studies) and beneficial effects in the treatment of glaucoma. It has also been shown to release various neutrophins, including BDNF, CNTF and b-FGF (*50*). Brimonidine belongs to the α-2 adrenergic receptor agonists. It is currently available as an ophthalmic solution (Alphagan-P, Allergan) for lowering the intraocular pressure in patients with open-angle glaucoma or ocular hypertension. A brimonidine tartrate intravitreal implant using the Allergan Novadur™ posterior segment drug delivery system is currently under evaluation in a Phase 2 study in regard to its efficacy and safety, and its possible effect on the progression of GA due to AMD (NCT00658619, ongoing, but not recruiting: 200 µg and 400 µg brimonidine tartrate posterior segment drug delivery system at day 1 and month 6). The implant delivers the drug to the retinal tissue over a period of 3 months.

AL-8309B (Tandospirone, Alcon Laboratories) (1.0% and 1.75% ophthalmic solution) is a selective agonist of the serotonin receptor ($5HT_{1A}$) that has been shown to protect the retina from severe photo-oxidative stress (*51*). AL-8309 was previously reported to upregulate antioxidant defense mechanisms in the retina (*52*) and to interfere with the complement pathway, preventing the deposition of complement C3, factor B, factor H, and membrane attack complex (MAC) (*53*). AL8309B was evaluated in a randomized, double-masked, multicenter, placebo-controlled GATE clinical study, as a topical ocular treatment for GA secondary to AMD. No positive results have been reported up until now.

The recently discovered rod-derived cone viability factor (RdCVF) which has been shown to induce cone survival and prevent secondary degeneration in cone photoreceptors in retinitis pigmentosa could also hold therapeutic promise in dry AMD (*54,55*).

MC-1101 (MacuCLEAR, Inc., TX, USA) is a novel, topically administered (eye drops, 1.0% migrating to the back of the eye) compound which increases ocular blood flow in the choroidal vessels and prevents progression of AMD from the early-stage dry AMD to the late-stage wet AMD. It is also believed to possess anti-inflammatory and antioxidative properties, and to reduce accumulation of retinal related waste by-products. The active ingredient of MC-1101 has been previously approved by the FDA as an oral antihypertensive drug with a well-characterized safety and tolerability profile. In April 2012, MacuCLEAR, Inc. announced that it is beginning Phase 3 studies for MC-1101 for early-stage AMD (60 patients) based on a successfully completed Phase 1b/proof of concept human clinical trial.

3.4. *Visual cycle modulators and drugs reducing the accumulation of toxic waste products*

The regeneration of the visual pigment 11-*cis*-retinaldehyde (11-*cis*-RAL, isoretinoin) from all-*trans*-RAL constitutes an important pathway in the visual cycle. Agents that slow this regeneration process (termed visual cycle modulators) decrease the accumulation of the toxic metabolites A2E and lipofuscin and may find therapeutic application in GA and other forms of macular degeneration.

Fenretinide (RT-101, ReVision Therapeutics, Inc., CA, USA) [4-hydroxy(phenyl) retinamide] is an oral synthetic retinoid derivative which down-regulates photoreceptor metabolism. It binds retinol-binding protein (RBP) in the circulation, blocks association between retinol and RBP, and prevents transport of retinol to the RPE. In 2007, Sirion initiated a randomized double-masked, placebo-controlled, dose ranging (100 and 300 mg/day) Phase 2 study (NCT00429936, completed) to evaluate fenretinide efficacy in patients with GA associated with dry AMD. A reduction in the incidence of wet AMD in patients with GA and slowing the growth of the GA lesions were communicated, but data were not accepted for review by the FDA and further research seems to be halted.

Another visual cycle modulator is the oral agent ACU-4429 (Acucela). ACU-4429 is a small non-retinoid molecule that functions as a modulator of the isomerase (RPE65) required for conversion of all-*trans*-retinol to 11-*cis*-RAL in the RPE (*56*). By modulating isomerization, ACU-4429 slows the visual cycle in rod photoreceptors and decreases accumulation of toxic fluorophores (A2E) and lipofuscin. It was demonstrated to completely prevent light-induced acute retinal degeneration in mice (*57*) and atrophic changes in the *Rdh8*(-/-)*Abca4*(-/-) retina (*58*). A single orally administered dose of ACU-4429 in healthy subjects produced a dose-dependent inhibition of the b-wave of the electroretinograms and was well tolerated up to 75 mg (*59*). A safety and tolerability clinical study of repeat doses (orally once a day for 14 days) of ACU-4429 in healthy subjects has been successfully completed demonstrating that this agent effectively targeted the visual cycle in a dose-dependent manner. An ongoing NCT01002950 Phase 2 study of the safety, tolerability, pharmacokinetics and pharmacodynamics of ACU-4429 in subjects with GA is underway. This is a multicenter, randomized, double-masked, placebo-controlled, dose escalation, multiple-dose study in which tablets (2, 5, 7, 10 or 20 mg) are taken orally once daily for 90 days. Because the drug is a non-retinoid, it may be potentially safe for a wide range of people, including young patients and women of child-bearing age. ACU-4429 may represent a new approach to treating dry AMD and other degenerative eye diseases, *e.g.* Stargardt disease.

RN6G (PF-4382923, Pfizer, New York, USA) is a humanized monoclonal antibody that targets the C-termini of amyloid beta-40 and amyloid beta-42. A safety and tolerability study of RN6G of single escalating doses (ranging from 0.3 mg/kg up to a maximum of 40 mg/kg intravenously) was completed in 2011 (NCT00877032). A multiple escalating dosages study (NCT01003691) is currently recruiting participants. GSK933776 (GlaxoSmithKline) is another humanized monoclonal antibody that decreases the levels of amyloid beta. It is currently under clinical investigation in a Phase 2 study for safety and efficacy in adult patients with GA secondary to AMD (CT01342926). Evaluation of this compound in patients with Alzheimer's disease is also underway.

3.5. *Drugs to suppress inflammation* (*complement inhibitors, immunomodulators and anti-inflammatory drugs*)

As mentioned above, the complement system is at the

core of many inflammatory processes. The presence of complement factor proteins in drusen in AMD eyes and genetic variation in several complement factor genes in individuals with AMD strongly support the involvement of the complement system in AMD predisposition and progression (*60-62*). To explore this etiopathological pathway towards development of therapeutic options for AMD, complement inhibitors, immunosuppressive agents and glucocorticoids are under extensive investigation.

3.6. *Complement inhibitors*

POT-4 (Potentia Pharmaceuticals), a synthetic 13 amino acid cyclic peptide, is a compstatin derivative that effectively inhibits the complement cascade by preventing cleavage of C3 (a central component of all three known complement activation pathways) to its active fragments C3a and C3b. Inhibition of the complement cascade at this level, including the C3 convertases, is of particular interest. This is because both amplification of all initiation pathways and generation of anaphylatoxins (C3a, C5a) and the membrane attack complex (MAC) are affected. This results in prevention of local inflammation, tissue damage and upregulation of angiogenic factors, such as vascular endothelial growth factor (VEGF) in the eye. In experimental models, compstatin has demonstrated effective complement inhibition with negligible toxicity (*63*). Suppression and reversal of drusen formation in monkeys with early-onset macular degeneration after 6 months of intravitreal injection of compstatin was reported (*64*). POT-4 recently completed a Phase 1 (ASaP) clinical trial (NCT00473928, safety of intravitreal POT-4 therapy for patients with neovascular AMD, single intravitreal injection). It was found to be safe and demonstrated definite biologic activity at the higher doses.

Another complement component inhibitor of possible therapeutic interest in dry AMD is the pegylated, aptamer-based anti-C5 agent ARC1905 (Archemix Copm.). ARC1905 inhibits cleavage of C5 into C5a and C5b and prevents formation of the key terminal fragments responsible for tissue pathology (*65*). These include the pro-inflammatory C5a and the membrane attack complex (MAC, C5b-9) which initiates cell lysis and releases proangiogenic molecules (*e.g.* PDGF and VEGF). MAC has been documented on the RPE, choroidal blood vessels, and drusen of AMD eyes. By inhibiting C5-mediated inflammatory and MAC activities, therapeutic benefit may be achieved in both dry and wet AMD. A randomized Phase 1 safety study (NCT00950638) of ARC1905 given as intravitreal injection is currently ongoing in subjects with dry AMD.

Eculizumab (Soliris, Alexion) is another anti-C5 drug. It represents a humanized monoclonal antibody derived from a murine anti-human C5 antibody. Eculizumab is the first FDA-approved complement inhibitor for the treatment of paroxysmal nocturnal hemoglobinuria. A Phase 2 randomized double blind clinical study "Complement Inhibition With Eculizumab for the Treatment of Non-Exudative Macular Degeneration" is underway (NCT00935883). The protocol aims at evaluating the change in drusen volume and area of GA. In the induction period, 600 mg or 900 mg eculizumab will be administered *via* intravenous infusion over approximately 30 min, once a week (7 ± 2 days), for 4 weeks. This will be followed by 900 mg or 1,200 mg eculizumab for the fifth dose 7 days later (7 ± 2 days). In the maintenance period patients will receive eculizumab 900 mg or 1,200 mg (intravenous infusion) over approximately 30 min every 2 weeks (14 ± 2 days) until week 24. Patients will further be observed for 6 months off treatment with follow-up visits scheduled for 9 months and 12 months.

TT30 (Taligen Therapeutics) is a new fusion protein coupling domains from complement receptor 2 (CR2) with the alternative pathway inhibitor factor H. It selectively binds to complement activated cells to locally regulate the complement system. Factor H defects or deficiencies can result in aberrant complement system activation and are associated with diseases such as atypical hemolytic uremic syndrome (aHUS) and AMD. It has been demonstrated that intravenously administered TT30 localized to the neovascularization lesion sites in mouse RPE-choroid, prevented the progression of choroidal neovascularization and ameliorated the diminished retinal function (*66*). TT30 is currently in a Phase 1 clinical trial "Safety and Pharmacokinetics of TT30 in Subjects With Paroxysmal Nocturnal Hemoglobinuria (PNH)" (NCT01335165). Taligen is also developing an intraocular injectable formulation of TA106 (a humanized anti-CFB monoclonal antibody fragment) that could be positioned for the AMD market. Another complement pathway-modulating compound currently being considered for possible use in AMD includes FCFD4514S, an anti-factor D (NCT01229215, Phase 2 ongoing).

Receptor antagonists comprise a new class of promising small molecules in the management of dry AMD. Compared with complement inhibitors that prevent the formation of the pro-inflammatory C5a fragment, these compounds competitively bind to the C5a receptor. Therefore, they have the potential to suppress the inflammatory response without affecting the protective complement-related immune responses. JSM-7717 and JPE-1375 (developing products of Jerini AG) are two peptidomimetic C5a receptor antagonists currently in preclinical assessment for AMD (*67*). Preclinical studies have suggested that JSM-6427, a potent and highly specific integrin α5β1 antagonist, may prevent conversion of dry AMD

to wet AMD and a dose-dependent inhibition of choroidal neovascularization in monkey and rabbit experimental models has been reported (*68*). A Phase 1 study was designed to evaluate safety, tolerability and pharmacokinetic profile of single and repeated doses of JSM-6427 in weekly intravitreal injections for up to 4 weeks to treat AMD (NCT00536016, completed, clinical results are still not published). Several additional complement pathway-modulating drugs are currently under evaluation in AMD, including CR2-CFH hybrid proteins, antiproperdin antibodies (thought to destabilize the critical C3 convertase), C1-INH and neutrazimab (classical pathway inhibitors), sCR1 (a soluble form of endogenous complement receptor which promotes the degradation of active C3bBb) (*65*).

3.7. *Immunosuppressive agents and steroids*

Copaxone (glatiramer acetate), a mix of four naturally occurring amino acids, L-glutamic acid, L-alanine, L-tyrosine, and L-lysine, is a synthetic protein that blocks the T-cell associated autoimmune responses and is approved for the treatment of multiple sclerosis. In a prospective interventional clinical trial of patients with dry AMD, treatment with copaxone for 12 weeks reduced drusen; this effect was confirmed by high-resolution spectral domain OCT/SLO (*69*). Copaxione is currently in Phase 2/3 clinical trials for dry AMD (NCT00466076). Neurodegenerative and inflammatory conditions, such as Alzheimer's disease, Crohn's disease, and acute optic neuritis are also in the spectrum of potential therapeutic indications for this compound.

Sirolimus (also known as rapamycin, Macusight/Santen) was originally developed as a macrolide antifungal agent. It inhibits T-lymphocyte activation and proliferation in response to both antigenic and cytokine stimulation, as reviewed (*70*). Due to its impressive anti-tumor, immunosuppressive and anti-angiogenic properties, sirolimus was approved for prophylaxis of organ rejection in renal transplants. The mechanisms of action of sirolimus imply inhibition of the mTOR-mediated signal-transduction pathways and associated modulation of cell growth, proliferation, motility, survival, protein synthesis, and transcription. It also modulates the activity of numerous survival proteins involved in angiogenesis and hyperpermeability, including VEGF. A Phase 1/2 randomized trial (NCT00766649) is currently ongoing to evaluate safety/efficacy of sirolimus to treat GA associated with AMD.

Steroids are used in single or combination therapy for AMD and other ocular diseases, such as diabetic macular edema, uveitis, and retinal vein occlusion. Currently, intravitreal administration of 0.2 and 0.5 μg/day fluocinolone acetonid (Iluvien, Alimera Sciences) is an object of a Phase 2 randomized, double-masked, fellow-eye comparison study (NCT00695318). It will evaluate the safety of fluocinolone and its efficacy to slow progression of GA in subjects with bilateral GA.

A newly emerging mechanism that has recently been linked to RPE degeneration and GA involves the decreased activity of DICER1, a microRNA-processing enzyme (*71*). DICER1 activates the NLRP3 inflammasome which was originally proposed to be a sensor of external danger signals, *e.g.* microbial toxins, and one of the key components of innate immunity. It was later found to be activated in diseases such as gout, atherogenesis, Alzheimer's disease, and type 2 diabetes. Targeting this pathway in GA *via* overexpression of DICER1 *e.g.* a vector-based approach to localized delivery of the DICER1 gene and/or antisense oligonucleotides against Alu RNA to regions of GA may ameliorate the disease and be a future therapeutic approach in AMD (*29*).

3.8. *Emerging approaches*

Cellular replacement strategies are believed to have the potential for restorative therapy for retinal degenerative diseases and AMD, and a considerable number of experimental studies in this direction has been undertaken. At least two mechanisms are expected to enable the visual improvements potentially associated with this therapeutic approach: a trophic effect of the implant on host cones and/or diffusion of soluble factors produced by healthy transplanted RPE cells to prevent progression of the disease (*7,72*) and local synaptic connections between the implant and host retina (*73*). RPE transplantation in human eyes with terminal AMD was performed initially in 1991 (*74*). So far, limited clinical trials of retinal implantation have been set.

Cell-based therapies represent a regenerative therapeutic approach that consists of introducing new cells to treat a disease. Both embryonic stem cell and induced pluripotent stem (iPS) cells are under extensive evaluation in retinal degenerative disorders. Differentiation of human iPS cells into RPE (*75*) has already been demonstrated and confirmed that iPS cells can generate functional iPS-RPE. Transplantation of these cells was shown to facilitate the short-term maintenance of photoreceptors through phagocytosis of the photoreceptor outer segments and to support long-term visual function. A secondary protective host cellular response has been suggested. Transplantation of iPS cell-derived RPE into rat models of retinitis pigmentosa has been shown to maintain visual acuity (*75*). So far, functional results with RPE transplantation techniques do not approach the levels of outcome seen with anti-VEGF treatment; in addition, there is a risk for nonterminally differentiated cells in the stem cell-derived RPE transplant to become tumorigenic (*76*). The value of stem cell-derived RPE transplantation is currently under evaluation: a Phase1/2 ongoing clinical trial (NCT01344993) assesses the effect of

transplantation of human embryonic stem cell (hESC)-derived RPE cells (Advanced Cell Technology) in retinal degeneration. Transplantation of fetal stem cells may have certain advantages (*e.g.* high immunologic tolerance and high capacity to produce trophic substances enabling the retinal connections) but it is also associated with some unfavorable conditions and ethical concerns. A Phase 1 clinical study (NCT01226628) of the safety and efficacy of the subretinal administration of human umbilical tissue-derived cells (hUTC; CNTO2476) in patients with GA has recently been initiated. The primary outcome measure will be the proportion of eyes with serious ocular adverse events occurring over the first 12 months of the trial. Secondary endpoints include evaluation of the clinical response (*e.g.* visual acuity) and the findings of OCT and fluorescein angiography (*e.g.* evolution of GA lesion size).

Optogenetics represents one of the newest strategies to restore vision and is currently under pre-clinical evaluation. Advantages of this therapeutic approach and the possible combination with other vision restoration methods were recently reviewed by Busskamp V *et al*. 2011 (*77*). Strategies that are practically feasible today are those using ubiquitous promoters to express optogenetic tools in retinal ganglion cells and those targeting the remaining degenerated cones using photoreceptor-specific promoters. The main advance of the optogenetic approach is that it may provide artificially stimulated retinal activity closer to the normal activity of retinal circuits.

The concept of retinal prostheses has been developed to restore useful vision in blind patients by activating the remaining inner retinal network (*78*). Many groups worldwide are working today on different types of retinal implant devices. At present, the US company Second Sight Medical Products has the longest and largest clinical follow-up with the epiretinal implant Argus™ designed to stimulate the underlying retinal ganglion cells. The first clinical trial with a 16-electrode device (Argus I) began in 2002 in 6 volunteers with advanced retinitis pigmentosa and reported encouraging results (*79*). A large-scale multicenter Phase 2/3 clinical trial is currently underway to evaluate a second generation implant consisting of 60 electrodes (Argus II) in 30 patients with profound visual loss due to retinitis pigmentosa. In the future, this device could possibly also be proposed as a therapeutic approach for patients with dry AMD.

4. Conclusion

The prevalence of AMD is expected to double in the next 20 years as the population ages. Given that there is no effective or approved treatment for dry AMD and no means to reduce the risk of a switch from slowly progressive dry AMD to an advanced and rapidly blinding wet AMD, developing new agents and treatment strategies should play a prominent role in the prevention and treatment of this blinding disease. Any advancement in this respect would bring enormous benefit to the patients that without treatment will gradually loose their vision, and with this, their independence to perform everyday tasks. Thus saving sight in an aging population is of major health, social and economic impact.

Although not exhaustive, the current review clearly shows that dry AMD has become a focus of the pharmaceutical industry for intervention and innovations, and that the pathway-based therapy holds a great therapeutic promise. Any of the complex mechanisms implicated in the etiopathological pathway of AMD could serve as a potential target for treatment. Regardless of the fact that at each step in AMD pathogenesis a new treatment could be developed (targeting oxidative stress, inflammation, impaired perfusion, cellular degeneration, accumulation of toxic metabolites, and so on), a complete inhibition of disease progression most likely will require a combination of different treatments and various approaches (*80*). Most of the treatment approaches and therapeutic agents described here are still under development and investigation, but the future holds a clear therapeutic promise for new therapies that may prevent or retard AMD progression and restore vision.

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Review

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Pseudomyxoma peritonei as an intractable disease and its preoperative assessment to help improve prognosis after surgery: A review of the literature

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Summary **Pseudomyxoma peritonei (PMP) is a rare and intractable disease with an estimated incidence of one per million population per year. Many aspects of PMP need to be fully and precisely understood; these include its preoperative assessment, *i.e.* diagnosis, early diagnosis, pathologic classification, and staging according to the peritoneal cancer index, and its surgical treatment. This review focuses on elements of preoperative assessment and surgery using the Sugarbaker procedure to help improve the prognosis for patients with PMP. Accurate data on the incidence of PMP must be based on large populations rather than estimates, and much work needs to be done especially in China. Special attention should be paid to its preoperative assessment. Also proposed here are steps to manage PMP with an emphasis on preoperative assessment.**

Keywords: Pseudomyxoma peritonei, preoperative assessment, prognosis

1. Introduction

Pseudomyxoma peritonei (PMP) is a rare and intractable disease. In 1884, Werth coined the term "pseudomyxoma peritonei" (*1*), which is characterized by excessive mucinous ascites and mucinous peritoneal implants, leading to progressive obliteration of the peritoneal cavity and intestinal obstruction (*2,3*). The accumulation of mucinous ascites and mucin-secreting epithelial nodules within the peritoneal cavity commonly results from the intraabdominal spread of invasive or non-invasive appendiceal tumors, and occasionally mucinous tumors at other sites, such as the colon (*4,5*) and ovaries (*6-8*) are responsible (*9*). PMP has frequently been classified as benign because it is almost noninvasive since it causes few lymphatic metastases and no hematogenic dissemination. However, the behavior of PMP over time suggests that it should be considered a borderline malignant condition with inevitable disease progression and a final terminal outcome. Recently, PMP has been referred to as a syndrome because of its different pathological types (*10,11*).

The clinical course of PMP is dictated by the volume of extracellular mucin that has accumulated and the degree of epithelial cellular atypia. Although PMP is less malignant and has a long clinical course, a radical resection is difficult and its prognosis is poor.

Because of its rarity, the incidence of PMP has yet to be fully determined. At present, there are no precise international data on PMP incidence, though an estimate is approximately 2 per 10,000 laparotomies or one per million population per year, with women mostly affected (2 to 3 times more frequently than men) (*12-17*). According to national data based on population, the annual incidence of PMP is 1,500 cases in the United States of America (USA) (*18*) and approximately 27 cases or 1.7 to 2 per million per year in the Netherlands (*12,15*). The incidence in Asia is about one per million per year and is presumed to be about a quarter of that in USA (*19*) (the estimated incidence of PMP is shown in Figure 1).

The treatment of PMP has yet to be firmly

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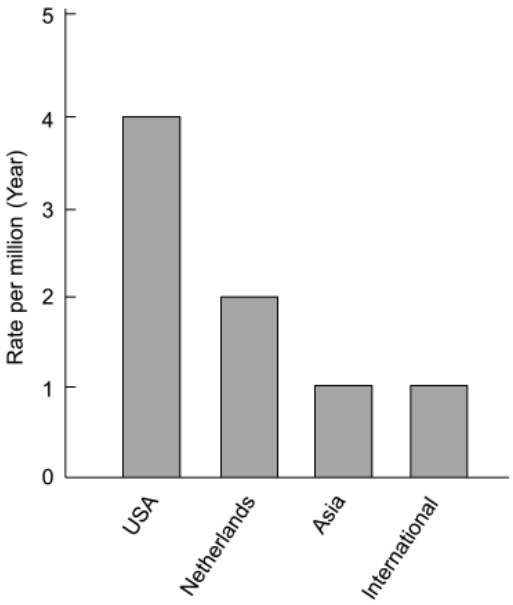

Figure 1. The estimated incidence of PMP (*Ref. 12-19*)**.**

established. Surgery is predominantly performed using the Sugarbaker procedure, but this procedure is controversial and not accepted globally. Clinicians lack a sufficient understanding of PMP and its surgical treatment in China. This review seeks to investigate surgery to treat PMP with a particular focus on its preoperative assessment.

2. Preoperative assessment: Diagnosis including pathologic classification and staging of PMP

Diagnosis, especially early diagnosis, is important to the success of surgery and prognosis for patients with PMP. Although rare, PMP covers a vast spectrum and has caused confusion over its identification and many errors in its diagnosis. The clinical manifestations of PMP are poorly defined due to few reports of large samples. Most patients are diagnosed during or after a laparotomy or laparoscopy for suspected appendicitis, peritonitis, or gynecological cancer. Wang *et al*. reported that patients in China are often misdiagnosed as having a malignant ovarian tumor, tuberculous peritonitis, or ascites resulting from liver cirrhosis; the misdiagnosis rate ranges from 18.8-100% according to the literature (*20-25*).

2.1. *Diagnosis with imaging assessment*

Imaging assessment is crucial to initial diagnosis. A CT scan is considered to be optimal for diagnosing PMP. (*26*). However, the diagnostic procedure often used first is ultrasonography. Typical ultrasound findings are non-mobile, echogenic ascites with multiple semisolid masses and scalloping of the hepatic and splenic margins (*27*). The results of a CT scan are sometimes pathognomonic findings that are considered to be highly suggestive of PMP (*28*). The most common findings on a CT scan are a large volume of mucinous ascites with the density of fat that displace the small bowel and the normal mesenteric fat. Other characteristic findings are omental thickening, multiseptate lesions, scalloping of organs, and curvilinear calcifications (*27-30*).

2.2. *Pathologic diagnosis and pathologic classification*

The pathologic classification of PMP is also key to surgical assessment and prognosis. There is considerable variability in the pathologic criteria and terminology used by different pathologists. For lesions with the same morphology, the diagnosis may be "a ruptured mucinous adenoma of the appendix and PMP" or "a ruptured mucinous adenoma of the appendix and PMP" according to different pathologists, especially in China. Most pathologists lack sufficient understanding of the pathologic classification of PMP (*21,31,32*). Ronnett *et al*. (*33*) proposed that the pathology of PMP be separated into 3 categories: *i*) low-grade tumors as disseminated peritoneal adenomucinosis (DPAM), *ii*) high-grade tumors as peritoneal mucinous carcinomatosis (PMCA), and *iii*) peritoneal mucinous carcinomatosis with intermediate or discordant features (PMCA-I/D). They reported that patients in these categories had survival rates that differed significantly, and results of long-term follow-up indicated significant differences in prognosis for DPAM and PMCA (*34*). Given these findings, therapeutic approaches can be rationally considered based on homogeneous pathologic entities. Findings also suggested that tumor tissue should be subjected to CK20 and CK7 immunohistochemistry. CK20 is a cytokeratin and intestinal tumor marker while CK7 is also a cytokeratin and marker of gynecological malignancies (*35*). The pathologic classification of PMP described thus far is gaining global acceptance (*36*).

2.3. *Staging of PMP according to the peritoneal cancer index score*

Another preoperative assessment that relates to treatment and prognosis is the staging of PMP. The peritoneal cancer index (PCI) scoring system is recommended for the staging of PMP. PCI has been used to assess the extent of the peritoneal spread of intraabdominal and intrapelvic malignant tumors. PCI provides valuable information about the exact distribution of seeding and tumor volume, representing in detail the extent of the peritoneal spread (*37-39*). PCI can help to determine treatment regimens and prognosis. PCI scoring is as follows (*37*): first, one of the thirteen abdominal regions should be scored. If there are no tumor nodules, the score is 0; if the largest tumor nodule is up to 0.5 cm in size, the score is 1; if

the largest tumor nodule is up to 5 cm, the score is 2; and if the largest tumor nodule is larger than 5 cm or tumors converge, the score is 3. Second, the scores for all thirteen regions are added together to yield the PCI score. Lower PCI scores are generally associated with a better prognosis and a greater likelihood of successful cytoreduction surgery. In some cases, a patient may have undergone surgery without intraperitoneal chemotherapy prior to cytoreduction surgery and intraperitoneal chemotherapy. The prior surgical score (PSS) gives a number value to surgeries done prior to the attempt at debulking/peritoneal chemotherapy treatment. However, Tentes *et al*. found that PSS was not related to survival for patients with PMP (*37*).

2.4. *Serum tumor makers*

In addition to clinical manifestations, imaging assessment, pathologic classification, and staging, several serum tumor markers, such as carcino-embryonic antigen (CEA), cancer antigen 125 (CA125), and carbohydrate antigen 19-9 (CA19-9), are also recommended to help diagnose PMP. The prognostic value of these tumor markers in patients undergoing surgery has been evaluated. Baratti reported that, according to univariate analysis, normal preoperative CA125 correlated with the likelihood of successful surgery and that, according to multivariate analysis, elevated baseline CA19-9 was an independent predictor of shorter progression-free survival (*40*). Van Ruth *et al*. (*41*) reported that elevated CA19-9 after surgery or during follow-up was related to disease recurrence.

3. Surgery to treat PMP

Since PMP is rare and intractable, patients with PMP have a poor long-term survival without definitive treatment, with 5-year and 10-year survival rates of 50% and 10-30%, respectively (*42*). Currently, there is no globally accepted standard treatment for PMP.

3.1. *Sugarbaker procedure*

The most satisfactory and effective treatment for PMP is surgical cytoreduction. Conventional surgical cytoreduction involved several surgeries until there were no further surgical options. Surgical debulking and appendectomy are widely regarded as the primary treatments for PMP. Sugarbaker developed and encouraged a complex approach involving cytoreduction and intraoperative hyperthermic intraperitoneal chemotherapy since the 1980s. A 5-year survival rate of 86% has been reported for some patients (*43*). Currently, the optimal therapy is complete macroscopic tumor removal (complete cytoreduction) combined with heated intraoperative intraperitoneal chemotherapy. Known as the Sugarbaker procedure, this treatment is considered to be the standard of care for PMP due to a perforated tumor in the appendix particularly in USA and Europe. In cases where complete cytoreduction cannot be achieved, maximal tumor debulking can be utilized (*44-49*).

While the Sugarbaker procedure has been adopted globally and is considered the optimal treatment for PMP, an optimal or standard treatment for PMP has yet to be established in China. There are few reported cases of Chinese patients undergoing complete cytoreduction combined with heated intraoperative intraperitoneal chemotherapy, and some clinicians complained of the lack of a unified standard for radical surgery (*3,5,50*). Kojimahara and Kitai *et al*. reported that radical cytoreduction and heated intraoperative intraperitoneal chemotherapy are not widely used in Japan. Given the risks of treatment, the procedure should ideally be performed at a referral center or at least by an experienced surgeon. An optimal or standard treatment for PMP has yet to be established in Japan (*51,52*).

3.2. *Sugarbaker procedure and its association with the CC score, morbidity, and mortality*

Details on the Sugarbaker procedure (*53-55*) are shown in Figure 2. Intraoperative hyperthermic intraperitoneal chemotherapy is used in combination with cytoreduction surgery to kill microscopic cells released into the peritoneal cavity from tumors during surgery or to kill cells released into the abdomen in cases of appendiceal rupture. Immediate assessment at the end of the surgery using the Completeness of Cytoreduction (CC) score (Table 1) is recommended. This scoring is crucial to assess prognosis. Given the complexity of the procedure, its morbidity and mortality are considerable. Major morbidity is considered to include anastomotic leakage, enteric and

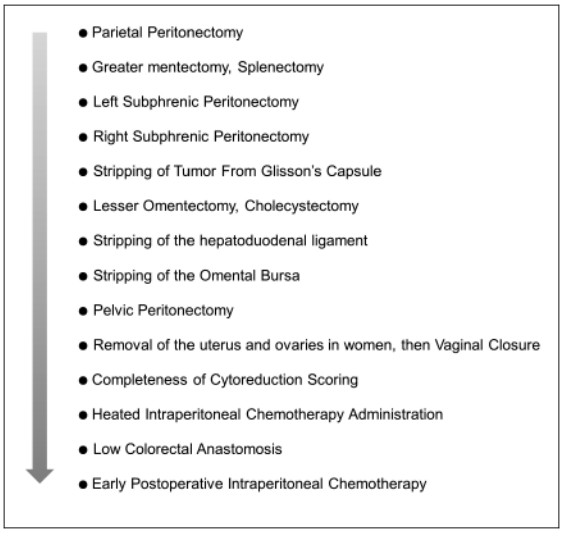

Figure 2. Chart of steps for the Sugarbaker procedure.

Table 1. Completeness of Cytoreduction (CC) Score (*Ref. 53*)

Score	Completeness of Cytoreduction
CC-0	All tumors are removed during cytoreduction surgery, and there is no visible cancer in the abdomen at the completion of the surgery.
CC-1	Tumor nodules remain in the abdomen or pelvis after surgery but are less than 2.5 mm in size.
CC-2	Tumor nodules remain in the abdomen or pelvis and are between 2.5 mm and 2.5 cm in size.
CC-3	Tumor nodules greater than 2.5 cm or a confluence (merging) of non-removable tumor nodules remain at any site in the abdomen or pelvis after surgery.

pancreatic fistulation, pneumonia, thromboembolism, and intra-abdominal abscesses (*21*). Mortality rates range between 0% and 14% (56). However, Youssef *et al*. (*57*) recently reported that the Sugarbaker procedure can be performed with a mortality rate below 2% and excellent long-term outcomes can be achieved in specialized units.

4. Patient eligibility for the Sugarbaker procedure

The Sugarbaker procedure has a relatively high morbility and mortality, so many clinicians wonder about the patient benefits of the Sugarbaker procedure. The benefits of this procedure must be evaluated in terms of the risks involved. According to a report (*58*) by Akshat Saxena *et al*., patients who were < 80 years old, with good performance status (World Health Performance Status B2), and adequate hematological, hepatic, cardiac, and liver function were eligible. Patients with extra-abdominal metastasis were ineligible. They also found that patients with an American Society of Anesthesiologists (ASA) grade of 3 or higher were significantly more likely to have severe complications and that perioperative mortality was significantly associated with a peritoneal cancer index (PCI) > 24. In detail, the ASA grade is as follows: a normal healthy patient is regarded as ASA grade 1, a patient with mild or severe systemic disease is regarded as ASA grade 2 or 3, a patient with an incapacitating systemic disease that is a constant threat to life is regarded as ASA grade 4, and a moribund patient who is not expected to survive for 24 hours without the operation is regarded as ASA grade 5 (*59*). Sugarbaker (*53*) reported that asymptomatic patients with a small volume of peritoneal surface malignancies must be selected for combined treatment.

5. Discussion and prospects for the future

PMP is a rare syndrome but affects a larger number of patients. However, the international incidence of PMP remains unclear. The data are estimates, and few studies have examined the incidence of PMP in large populations, especially in China. The PubMed database and Chinese CNKI database revealed few studies at multiple centers or with large samples of Chinese patients. Therefore, a specific and specialized website should be created to collect information on and determine the incidence of PMP, and this website can help to educate doctors in China. A peritoneal malignancy treatment center should also be established in China to help treat PMP and rare and intractable conditions like it (*57,60*).

PMP is not only rare but also intractable, which is the cause of its poor prognosis. The treatment of PMP is controversial and lacking in hard scientific evidence. Such evidence is unlikely ever to be available due to the rare heterogeneity of the disease (*61*). Although controversial, the Sugarbaker procedure is gaining internationally acceptance as the standard treatment for PMP. Mortality associated with the Sugarbaker procedure can reportedly be reduced to less than 2% and excellent long-term outcomes can be achieved in specialized units (*57*), representing substantial progress in the treatment of PMP. The outcomes of initial surgery are significantly related to prognosis, so the preoperative assessment of PMP in relation to surgical outcomes should be comprehensively and carefully considered.

First, PMP should be diagnosed early. Since PMP involves unspecified clinical manifestations and ultrasonography is frequently performed as the initial diagnostic procedure, sonographers should be informed about PMP. Screening for serum tumor markers such as CEA, CA125, and CA19-9 is also suggested for early diagnosis. Surgeons and general physicians who diagnosis patients with "a malignant mucocele or malignant mucocele with peritoneal dissemination" must promptly refer those patients to a specialist (*57*). If these patients are promptly referred, surgery is less extensive, morbidity and mortality rates will be lower and hospital stays will be shorter, and long-term results will improve.

Second, preoperative pathologic classification of PMP must be precise and definite. However, the definition of PMP has been a source of much confusion, with different reports including patients with ovarian, colon and other primary tumors, in addition to appendiceal tumors (*62-64*). Thus, the pathologic description and classification of PMP is unclear. Most Chinese pathologists lack a sufficient understanding of the pathologic classification of PMP,

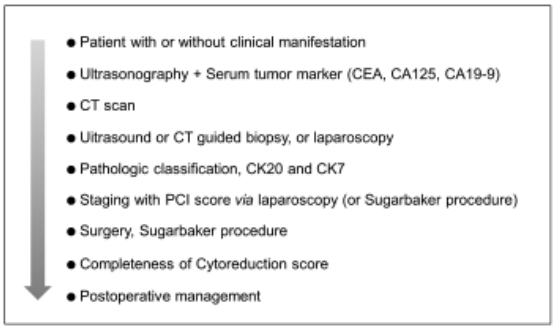

Figure 3. Proposed chart of steps in the management of PMP.

so PMP must be described according to a unified pathologic descriptive terminology. The currently accepted pathologic classification is the 3-category classification, *i.e*. DPAM/PMCA/PMCA-I/D, proposed by Ronnett *et al*. (*33*). This pathologic classification of PMP is significantly related to the treatment and prognosis of PMP, so it should be utilized in the pathologic classification of PMP. CK20 and CK7 immunohistochemistry should also be utilized. The question then is how to reach a preoperative pathologic diagnosis. A CT or ultrasound-guided biopsy or a laparoscopic examination and biopsy should be performed on patients suspected of having PMP.

The third point is the staging of PMP in accordance with the preoperative peritoneal cancer index (PCI). PCI is a clinical integration of both peritoneal implant size and distribution of nodules on the peritoneal surface. It is crucial to prognosis and also helpful in determining which patients with PMP are eligible for surgery. It should be used in the decision-making process as the abdomen is completely explored, but can the PCI be determined noninvasively before surgery? Is a CT PCI possible? Studies have unanimously concluded that CT sensitivity increases markedly with larger implants (*26,65*). The results of a study by Jacquet *et al*. are comparable to other studies: 28% for nodules < 0.5 cm in diameter compared to 90% for ones > 5 cm (*65*). Additionally, de Bree *et al*. found that CT rather inaccurately represented the actual size of peritoneal nodules (*66*). A CT scan's ability to detect peritoneal implants is influenced by lesion size and CT PCI significantly underestimates the clinical PCI. The mean operative PCI is nearly double that approximated by CT. A reasonable approach may be for patients with a preoperative CT PCI > 15 to be considered ineligible for combined treatment because their clinical PCI may be much higher (*67*). Thus, the preoperative PCI should be determined by laparotomy just before the Sugarbaker procedure or laparoscopy.

In conclusion, PMP is a rare and intractable entity. Special attention should be paid to its preoperative assessment, including early diagnosis, pathologic classification, and peritoneal cancer index. Proposed here are steps for managing PMP that include preoperative assessment (Figure 3). Currently, PMP is treated with complete cytoreduction combined with heated intraoperative intraperitoneal chemotherapy (Sugarbaker procedure) as a widely accepted and even standard curative treatment in Europe and the US. In Asia, however, this treatment is not as widely accepted as conventional surgery. If patients are appropriately selected, the Sugarbaker procedure can provide excellent long-term outcomes. In the absence of animal models or randomized controlled trials, further efforts should be made to obtain evidence and improve treatment outcomes for this challenging, though rare condition.

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Review

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Progress in the clinical imaging research of bone diseases on ankle and foot sesamoid bones and accessory ossicles

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Summary **Sesamoid bones and accessory ossicles are research focuses of foot and ankle surgery. Pains of the foot and ankle are related to sesamoid bones and accessory ossicles. The specific anatomical and functional relationship of sesamoid bones and accessory ossicles can cause such bone diseases as the dislocation of sesamoid bones and accessory bones, infection, inflammation and necrosis of sesamoid bones, cartilage softening, tenosynovitis of sesamoid bones and the sesamoid bone syndrome. However, these bone diseases are often misdiagnosed or mistreated. In patients with trauma history, relevant diseases of sesamoid bones and accessory ossicles as above mentioned are highly probable to be misdiagnosed as avulsion fractures. In such cases, radiographic findings may provide a basis for clinical diagnosis.**

Keywords: Ankle and foot, sesamoid and accessory bone, bone diseases, image

1. Introduction

Sesamoid bones and accessory ossicles of the foot and ankle, due to their large quantity and complex structure, play an important role in foot and ankle surgery and are attracting increasing and considerable attention of surgeons. Studies on such diseases as the dislocation of sesamoid bones and accessory bones, infection, inflammation and necrosis of sesamoid bones, cartilage softening, tenosynovitis of sesamoid bones and sesamoid bone syndrome are a few current areas of interest. Surgeons usually lack a reliable diagnostic basis for these diseases, and problems of misdiagnosis, mistreatment and missed diagnosis often occur. There are also the cases in which sesamoid bones and accessory ossicles are misdiagnosed as bone fractures. Particularly, with the accessory bones of the fibula and the bottom and accessory bones between the talus and the fibula, the rate of misdiagnosis of fractures is respectively 13.3% and 16.7%. Fracture of sesamoid bones can be misdiagnosed as bipartite or multipartite sesamoid bones, thus causing spiritual and economic loss to patients. Our study finds that magnetic resonance imaging (MRI) and bone scanning can help early and accurate diagnosis of infection and inflammation of sesamoid bones and accessory ossicles, necrosis of sesamoid bones, sesamoid bone syndrome, *etc.* According to the predilection sites of diseases of sesamoid bones and accessory ossicles as well as X-ray findings, sesamoid bones and accessory ossicles can be distinguished from an avulsion fracture.

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2. The mechanism of sesamoid bones and accessory ossicles

Sesamoid bones are embedded within tendons and ligaments. Accessory bones result from a non-combination of several ossification centers or development of extra and independent ossification centers. Such phenomena are usually found in the human foot and ankle with both sesamoid bones and accessory ossicles existing as ossicles. They have bone cortex and cancellous bone with a smooth and regular profile. In a tangential position, accessory bones and sesamoid bones are clearly separated from the surrounding bones.

3. Inspection methods of images

The ankle and foot joints were observed with

anteroposterior and lateral X-rays, from patients suffering sesamoid bones and accessory ossicle lesions that could not be distinguished from fractures. Dislocation of the sesamoid bones and fracture of accessory ossicles were further examined by contralateral radiographs or even axial radiographs of the sesamoid bones and accessory ossicles. Computed tomography (CT): the patient sat down, with feet together and flat, KV:120 and mA:250, layer thickness 1mm, and layer distance 1 mm, the scanning area ranged from the top of the articular surface of the second metatarsal bone to the highest point of the articular surface of the calcaneus. MRI scanning plane: axial (vertical to the connecting line between from the highest point of the articular surface of the calcaneus and the top of the articular surface of the second metatarsal bone on the sagittal scout image), coronal (the section of the second metatarsophalangeal joints section on the axial image was parallel to the plane of the 5 metatarsuses or the lower edge of the metatarsuses), sagittal (the position on coronal image was parallel to the long axis of the second metatarsus), scanning sequence: SE T1WI, GRE T2WI and ST IR. Horizontal axis plane and sagittal plane radiograph were usually adopted. Layer thickness 1 mm, interval 0-1 mm, and matrix: 512 × 512.

4. Imaging of bone diseases on ankle and foot sesamoid bones and accessory ossicles

4.1. *The dislocation of sesamoid bones and accessory ossicles*

Sesamoid bones and accessory ossicles dislocations often occurred after ankle and foot joint trauma, which was most commonly seen in the first metatarsophalangeal joints of the foot. Hyperextension of the first metatarsophalangeal joint often results in dislocation of the proximal phalanx on the head of the metatarsal bone (*1*). Thus adjacent sesamoid bones are dislocated to the outward lateral side, or the sesamoid bones might also dislocate to the proximal head of the metatarsal bone. Different degrees of sesamoid bone dislocations were seen in valgus, and they were significantly correlated with each other, the sesamoid bone dislocations became more significant as the HVA (hallux valgus angle) and MA (the angle between the first and second metatarsus) increased (*1*). The clinical symptoms were local soft tissue distention and positive tenderness. The imaging findings were significant distention of adjacent soft tissue, translocations of the sesamoid bones and accessory ossicles, which were usually accompanied by the rupture of tendons and ligaments, and the fracture of adjacent skeleton, *etc.* The patients would have a radiograph of the contralateral part for contrast diagnosis.

4.2. *Fracture of the sesamoid bones and accessory ossicles*

The sesamoid bones and accessory ossicles are prone to be misdiagnosed as an avulsion fracture in the case of trauma, however, fracture indeed sometimes occurs in the sesamoid bones and accessory ossicles. The diagnosis is mainly based on radiographs, while the sesamoid bones lesions are generally unclear in conventional radiographs (*2*). Additional axial radiographs of the sesamoid bones and accessory ossicles are necessary in which the broken ends of the fractured bone are sharp and irregular. Callus is seen forming surrounding broken ends in a follow-up examination, and the adjacent soft tissue is obviously swollen and accompanied with severe pain. Those suspect patients need to have a radiograph of the contralateral part for contrastanalysis (Figure 1), and they should be differentiated from bipartite sesamoid bones and tripartite sesamoid bones. Bipartite sesamoid bones and tripartite sesamoid bones are normal skeletal variations in which the edges are round, blunt and regular with a smooth and intact adjacent bone cortex; furthermore, the shape, size and position of

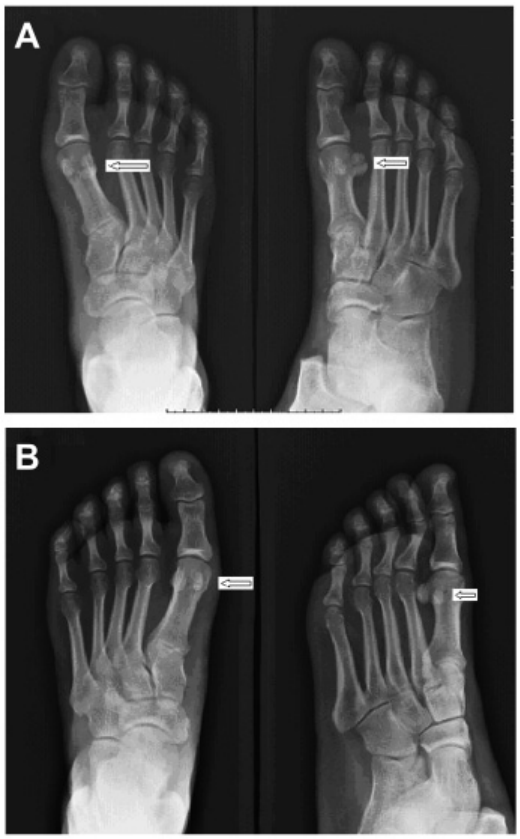

Figure 1. Fracture of the sesamoid bones. (A) Radiograph after trauma of the right foot fracture of the sesamoid bones on the outward lateral side of the first caput of metatarsal bone which is broken into 2 fragments, the broken end is sharp; **(B)** Sesamoid bone of the first caput metatarsalof the left foot of the same patient is normal.

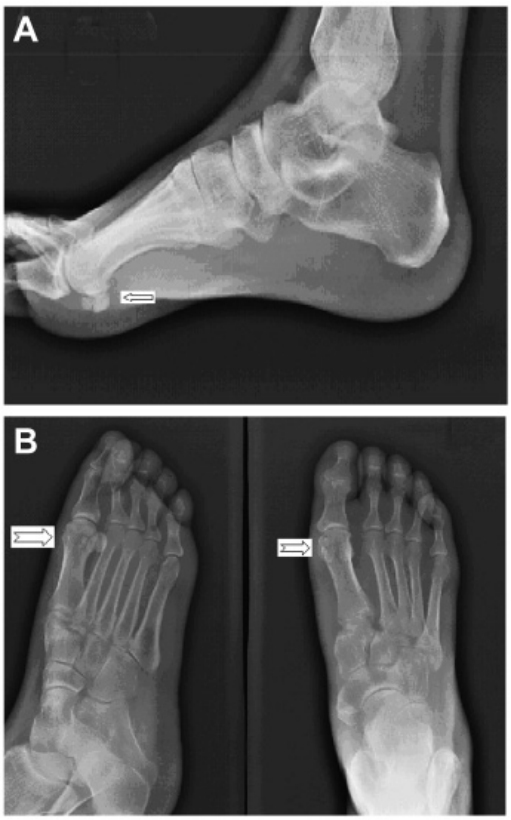

Figure 2. Multipartite sesamoid bones. (A) Bipartite sesamoid bones of the first metatarsus of right foot; **(B)** Multipartite sesamoid bones of the first metatarsus of right foot, the edge of each sesamoid bone is round, blunt and regular, and the adjacent bone cortex is smooth and intact.

the sesamoid bones in follow-up examination are unchanged (Figure 2).

4.3. *Inflammation of sesamoid bones and accessory ossicles*

Trauma is the primary cause of pathogenesisand most of the inflammations are induced by repetitive injuries, while once acute severe impingement can also cause fracture or inflammation of sesamoid bones or accessory ossicles. They mainly occur in the outward lateral side of the first metatarsophalangeal joints. Clinical symptoms include redness and distention of local soft tissue accompanied by significant tenderness. There is generally no characteristic change in radiographs, and MRI imaging shows distention and a fluid effusion shadow in the adjacent soft tissue. There is usually no significant abnormality in the sclerotin of sesamoid bones, and bone scanning shows the radionuclide concentration in the local sesamoid bones. Thus the diagnosis is primarily based on clinical symptoms and physical symptoms, and eventually confirmed by pathological examination (Figure 3).

4.4. *Sesamoid bones infection*

Sesamoid bones infection are usually disseminated from

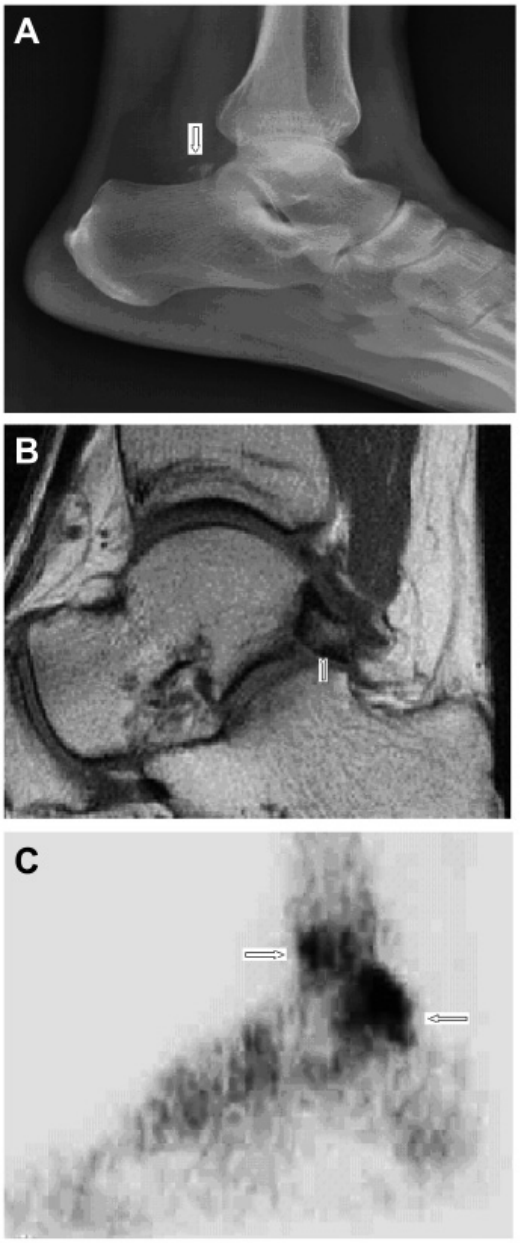

Figure 3. Inflammation of the sesamoid bones and accessory ossicles. (A) The accessory ossicle (OS trigonum) behind the talus of left foot and the sclerotin of accessory ossicle are normal; **(B)** T1W1: the small sesamoid bones over the calcaneus and behind the talus, low signal liquid shadow can be seen in the adjacent area, there is edema in the sesamoid bones and adjacent bone marrow is distended, and effusion is in the surrounding intervals; **(C)** Bone scanning: two radionuclide concentration areas can be seen in the lower end of shinbone and behind the talus.

adjacent tissue or by blood, and the latter situations are often seen in children and youth. Clinical symptoms include local soft tissue distention, increasing skin surface temperature and apparent tenderness. Radiographs show that the edge of the sesamoid bones is coarse, the density is inhomogeneous, and a low density destruction area of sclerotin can be seen. CT imaging shows distention of adjacent soft tissue and arthroedema. The early stage diagnosis can be made by MRI. MRI imaging shows scattered or irregular

strip sesamoid bones, a mixed signal of low, middle and high degree, and adjacent soft tissue distention and liquid effusion shadow. Bone scanning indicates radionuclide concentration in the local sesamoid bones (Figure 4).

4.5. *Necrosis of sesamoid bones and accessory ossicles*

Necrosis of sesamoid bones and accessory ossicles is more often seen in females, and usually is related to trauma. The clinical manifestations include apparent local tenderness. Radiographs show that the shape and outline of the sesamoid bones are irregular with inhomogeneous density (Figure 5). CT imaging shows that the sesamoid bones edge is coarse, density increases, and a linear and cystic translucent area can be seen within the sesamoid bones. MRI imaging has a diagnostic value for necrosis of sesamoid bones in

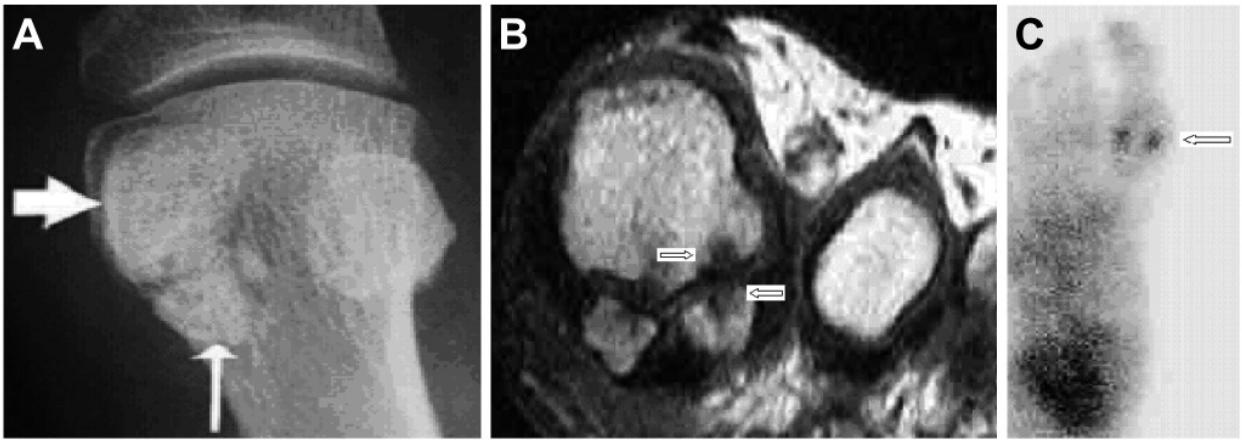

Figure 4. Sesamoid bones infection. (A) The edge of the sesamoid bones of first caput of metatarsal bone is coarse with inhomogeneous density; **(B)** T1WI: irregular low signal of first caput of metatarsal bone and sesamoid bones, joint chondromalacia, thickened flexor tendon of second metatarsus; **(C)** Bone scanning: radionuclide concentration in the first caput metatarsal of the feet.

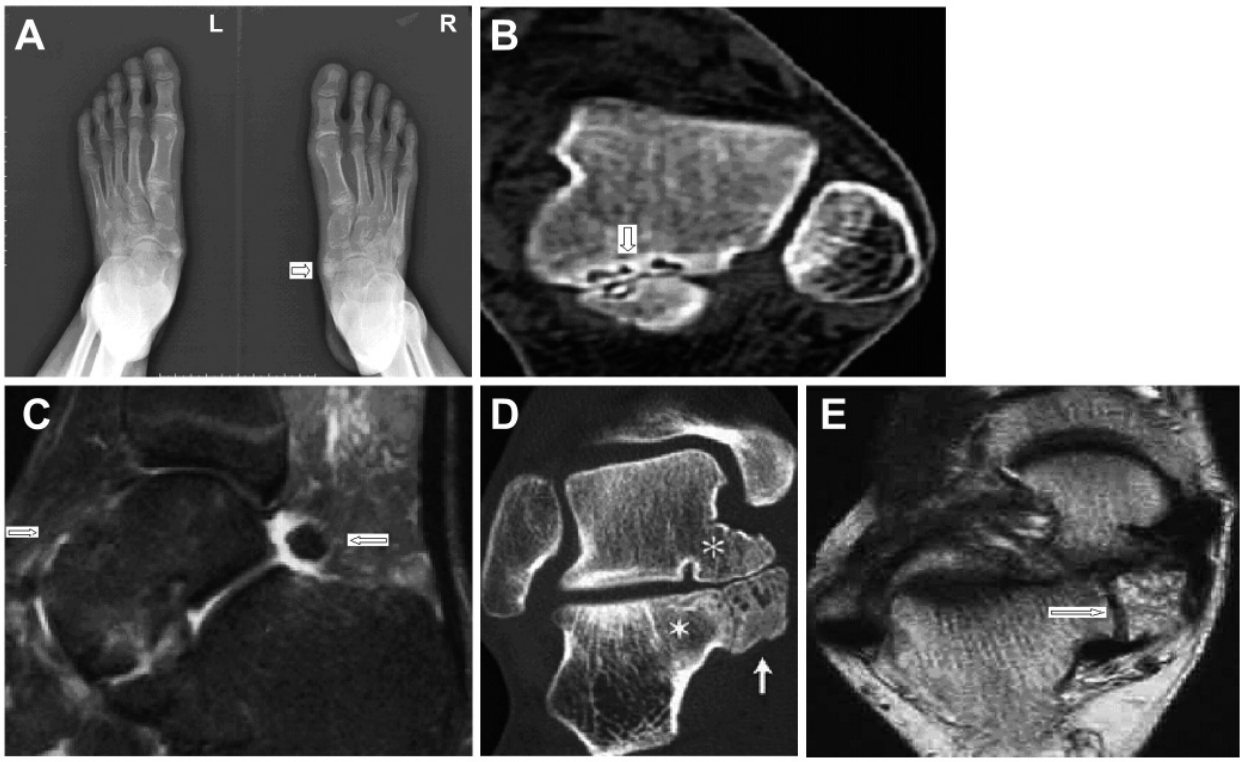

Figure 5. Necrosis of sesamoid bones and accessory ossicles. (A) The density of the articular surface formed by the navicular and accessory navicular of the right foot increases, the density of accessory navicular increases, small saccular translucent area can be seen at the edge of accessory navicular, there is no significant abnormality in the navicular and accessory navicular of left foot; **(B)** CT scanning of the posterior margin of the talus of another patient, there are several small saccular translucent areas in the margin of the OS trigonum and talus, the shape of the accessory ossicle is irregular; **(C)** MRI of OS trigonum, effusion signal shadow can be seen in adjacent soft tissue and can also be seen in the interval space of the talonavicular joint; **(D)** The accessory ossicle in the medial margin of the calcaneus of another patient, there are several cystic lesions; **(E)** Accessory ossicle marrow edema.

the early stage, sesamoid bone marrow edema can be seen in the early stage which manifests as long T1 and T2 signals, and a long T1 and short T2 cystic necrosis area might appear within the sesamoid bones following development of illness.

4.6. *Sesamoid bones chondromalacia*

It is often seen in the articular surface of the first metatarsophalangeal joint, and most of them result from sesamoid bones degeneration induced by repetitive strain, and the clinical symptoms include local pain, while there is no distention in the adjacent skin area. Radiograph examination: radiographs show that the shape and edge of the sesamoid bones are irregular with an inhomogeneous density, a linear and cystic translucent area can be seen within the sesamoid bones area, and axial images show that the sesamoid bones surface is coarse and there are some bone fragments. MRI imaging shows scattered, or irregular strip sesamoid bones, a mixed signal of low, middle and high degree, bone marrow edema is visible under the sesamoid bones cartilages, and the dissociative bone fragments can also be seen in the adjacent area of the sesamoid bones (Figure 6).

4.7. *Tenosynovitis induced by sesamoid bones*

The formation of stenosal tendosynovitis is closely related to the sesamoid bones, the skeletal structures adjacent to the tendons. In particular, the sesamoid bones can cause or promote formation and development of stenosal tendosynovitis to some extent. The clinical symptoms of stenosal tendosynovitis include local distention, pain and a snap sound (*3-5*), radiographs showthat bone spurs form in the joints adjacent to sesamoid bones (*6*), and MRI imaging shows that there is effusion adjacent to sesamoid bones and visible liquid signal shadows within the tendons (Figure 7).

4.8. *Sesamoid bones syndrome*

Sesamoid bones syndrome refers to the deformation, crush, cystic change and proliferation of the sesamoid bones induced by repetitive impingement on sesamoid bones. The impingement will also increase pressure in the capsule of adjacent synovial joints, which might result in the repression of local soft tissue and a following inflammatory reaction, and then tenosynovitis of the flexor tendon, thickening and fibrosis of the joint capsule will be induced (*7*). The primary clinical symptom is acute joint impingement, which mainly includes local soft tissue distention, tenderness, asymmetric buckling of joints and stiff joints. Howse (*8*) has reported a relatively specific method to test the excitability which reproduces the corresponding symptoms by buckling as well as simultaneous rotating and impingement of the joints.

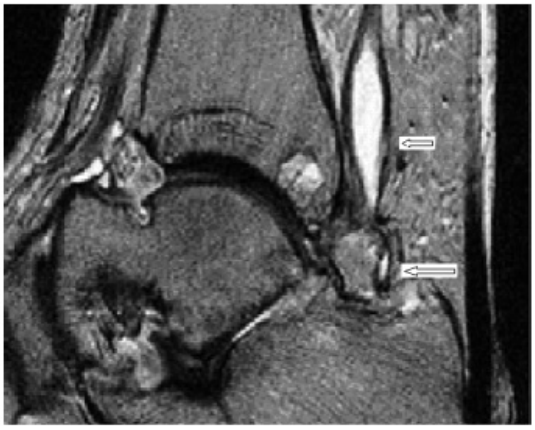

Figure 7. The tenosynovitis induced by sesamoid bones. T2WI: long T2 liquid signal shadow can be seen in the accessory ossicle (OS trigonum) above the calcaneus and behind the accessory ossicles and the flexor pollicis longus muscle tendon; there is pyema in the flexor pollicis longus muscle tendon.

Figure 6. Sesamoid bones chondromalacia. STIR sesamoid bones chondromalacia patient. There are some small dissociative bone fragments in the adjacent area of the sesamoid bones.

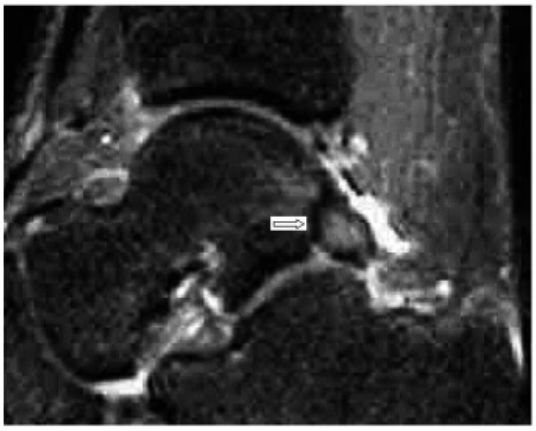

Figure 8. Sesamoid bones syndrome. STIR Low liquid signal shadow can be seen in the surrounding area of the accesssory ossicles behind the talus (OS trigonum) and above the calcaneus; there is edema in the sesamoid bones and adjacent bone marrow, and effusion in the adjacent interval.

Radiographs only show distention of the soft tissue adjacent to sesamoid bones, while the distention of the adjacent soft tissue and effusion in the articular cavity can be seen in CT imaging. Bone scanning shows the radionuclide concentration in the local sesamoid bones, and the sesamoid bones syndrome can be diagnosed by MRI imaging, in which the radiographs of the horizontal plane and vertical plane are usually adopted, and the vertical plane is particularly important (*9*).

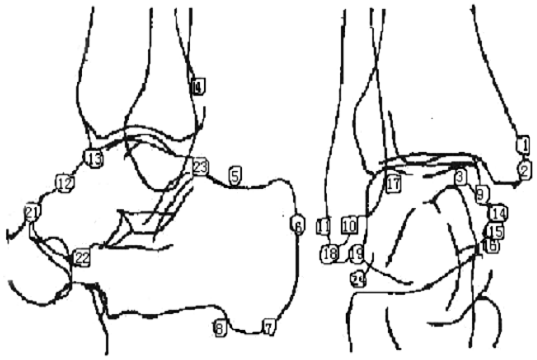

Figure 9. Site where accessory bone is existent. Generally, 1-8 are sites having no accessory bones. If bone fragments are existent at these sites, they are probable calcified or detached sclerites. Generally, 9-13 are the rarest sites where accessory bones are found. If there are bone fragments, they resulted from bone fracture in most cases. Generally, 14-23 are occasional sites where accessory bones are found. If there are bone fragments, the probability of bone fracture should be first excluded. Generally, 24 is the site where accessory bone is existent. However, bone fracture is also possible at this site.

Bone marrow edema, soft tissue distention and tendon laceration (*10,11*), and arthroedema (*12,13*) can be seen in ST IR sequence. T2WI shows mild thickening of aponeurosis adjacent to the sesamoid bones and degenerative cystic change signals at the connection area between sesamoid bones and cartilages, as well as myotenositis of the long flexor muscle of the thumb and effusion within its tendon sheath (Figure 8). Conservative treatment is the first choice, the sesamoid bones and accessory bones should be resected only when necessary (*14-16*).

4.9. *Identification of key points of sesamoid bones, accessory ossicles and avulsion fracture*

The edge of sesamoid bones and accessory ossicles is smooth, the density of adjacent cortical bone is high, the cortex is intact, the adjacent bone structures are intact and symmetrical, the shape and position of these structures in follow-up radiographs are unchanged, and generally there is nearly no pain (Figure 9). However, fracture usually has a definite history of trauma, adjacent soft tissue distention is significant with apparent pain, the cortex is broken, the broken ends are sharp and asymmetrical, and the shape and position of these structures might change in follow-up radiographs (Figures 10A-10F).

5. Conclusion

Many skeletal variations in the ankle and foot may

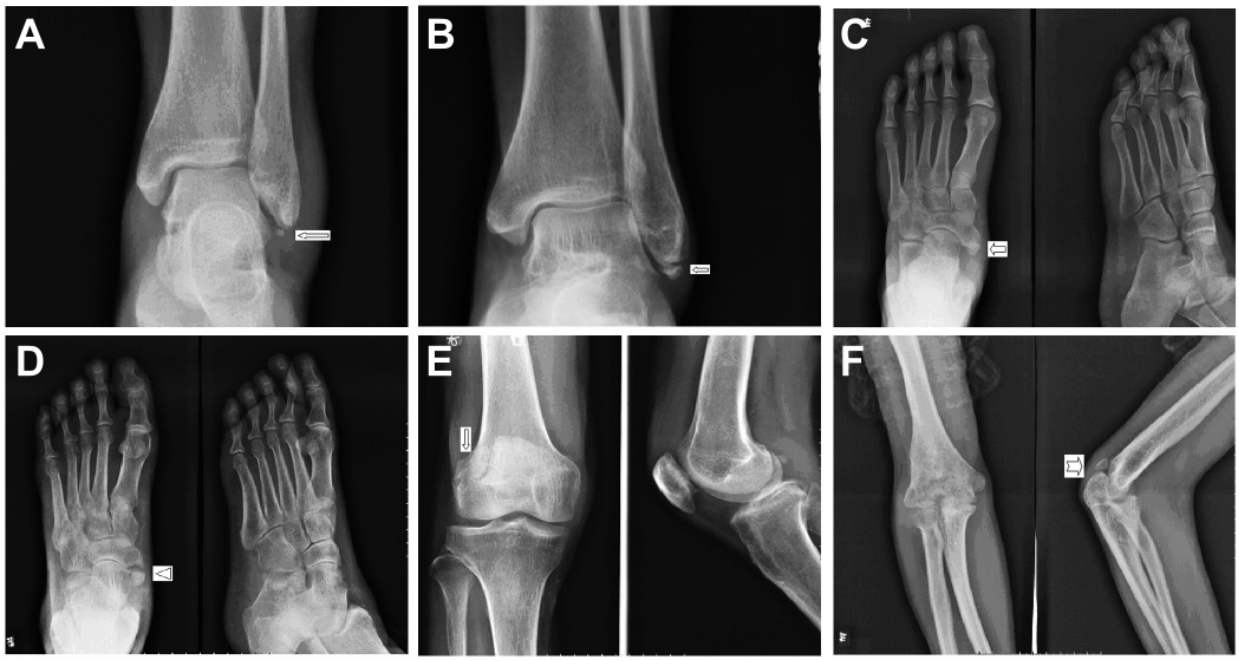

Figure 10. The identification of sesamoid bones, accessory ossicles, and avulsion fracture. (A) Os subfibulare in the lower end of extramalleolus: the soft tissue of os subfibular distends, while the bone edge is smooth, the density of the surrounding bone cortex increases, the shape and position of the bone mass is unchanged in the follow-up examination; **(B)** The fracture in the lower end of os subfibular: the fracture line is clear and sharp, the broken end is separated, and the adjacent soft tissue distends slightly; **(C)** Accessory navicular: the edge of the bone mass is smooth and the edge of navicular is regular; **(D)** Navicular fracture: the edge of the navicular is irregular and sharp; **(E)** and **(F)** are respectively bipartite patella and patella cubitus: the edge of the bone mass is smooth, the adjacent bone structures are intact.

be found, including different accessory ossicles and sesamoid bones, bipartitions and coalitions (*17,18*). Most accessory ossicles and sesamoid bones do not cause any complaints and remain asymptomatic. Generally, they are detected by routine radiologic examinations after trauma or overuse leading to degenerative changes or pain. They may also suffer or stimulate fractures and restrict the range of motion (*19-23*). In the literature reported, incidence of the accessory ossicles in the foot and ankle is 18-36.3% in the general population (*24*).

Sesamoid bones and accessory ossicles are research focuses of foot and ankle surgery (*25*). Pains of the foot and ankle are related to sesamoid bones and accessory ossicles (*26*). Clinical disease of the sesamoid bones and accessory ossicles of the ankle and foot joints is common in clinical practice, while the clinical symptoms and physical signs are not specific. Thus they are difficult to diagnose, and these bone diseases are often misdiagnosed or mistreated (*27-30*). The pathogenesis correlates with their anatomy and function, the comprehensive understanding of their imaging findings has important significance on the accurate diagnosis and treatment of these diseases.

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Review

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Role of duplex ultrasound in the diagnosis and assessment of carotid body tumour: A literature review

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Summary **Carotid body tumour is a rare disease, a slow growing highly vascular tumour of the carotid body tissue and the most common type of the paraganglioma. This article reviews the pathological, clinical and ultrasound features of carotid body tumours and discusses the role of duplex ultrasound in the diagnosis and assessment of this condition. The initial presentation of carotid body tumour is usually a painless palpable neck mass. Some patients may experience local pressure symptoms as well as symptoms from vagal, hypoglossal and cervical sympathetic nerve impingement. Percutaneous needle aspiration or incisional biopsy is contraindicated for the diagnosis of carotid body tumours. Duplex ultrasound, computed tomography scan, magnetic resonance scan and angiography are commonly used diagnostic tools for this condition. Complete surgical excision of carotid body tumour is the treatment of choice as radiation therapy and chemotherapy are unsatisfactory. Based on vascularity and location, duplex ultrasound scan is able to diagnose carotid body tumour and differentiate it from many other masses in the neck. This non-invasive, inexpensive and readily available diagnostic tool can be used as a first-line imaging modality for the diagnosis and assessment of carotid body tumours.**

Keywords: Carotid body tumour, ultrasound, literature review

1. Introduction

Carotid body tumour is a rare disease. It is estimated the incidence of carotid body tumour is about 1 in 30,000. Most vascular surgeons will encounter very few during their career (*1*). Angiography has been the gold standard for the diagnosis and management of carotid body tumours for many years. Duplex ultrasound has been increasingly used in the diagnosis and assessment of carotid body tumours since colour flow imaging was introduced and became a non-invasive alternative imaging modality. This article will review the pathological, clinical and ultrasound features of carotid body tumours and discuss the role of duplex ultrasound in the diagnosis and assessment of this condition.

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2. Carotid body

The carotid body was first described by Von Haller in 1743 (*2*). It is a chemoreceptor located in the adventitia of the carotid bifurcation. The size of a carotid body is approximately 5 mm × 3 mm × 2 mm. When the carotid body detects decreasing levels of oxygen (hypoxia), increasing levels of carbon dioxide (hypercapnia) and decreasing pH (acidosis), it increases respiratory rate, tidal volume, heart rate, and blood pressure together with vasoconstriction, and the production of circulating catecholamines (*3*).

3. Carotid body tumour

Carotid body tumour is hypertrophy of the carotid body tissue, which is the most common type of the paraganglioma. It also known as a chemodectoma, endothelioma, glomus caroticum, perithelioma, chromaffinoma and nonchromaffin paraganglioma. Carotid body tumours may be familial (10-50%) (*4*) or non-familial. The incidence of bilateral carotid body

tumours is 32% in the familial group (*5,6*), and 5% in the non-familial group (*7-9*). Most carotid body tumours are slow growing and benign. Approximately 5-10% of carotid body tumours may progress to malignancy, with local vascular and/or lymph node invasion, and rarely distant metastases (*9-12*).

3.1. *Pathology*

Macroscopically, carotid body tumours are well circumscribed, rubbery, and reddish brown. Microscopically, the tumours are highly vascular; between the many capillaries are clusters of cells, including supporting cells and chief cells. Cytochemical techniques usually demonstrate epinephrine, norepinephrine and serotonin in these cells (*13*). Unlike other neoplasms, malignant carotid body tumour does not have histological transformation (*9,13,14*). The diagnosis of malignancy is based on vascular/lymph node invasion and metastases.

3.2. *Clinical Presentation*

The initial presentation of carotid body tumour is usually a painless, palpable neck mass. Some patients may experience local pressure symptoms, such as neck or ear pain, sore throat, local tenderness and odynophagia. Symptoms from vagal, hypoglossal and cervical sympathetic nerve impingement, such as hoarseness, dysphasia, dysarthria, swallowing difficulties and Horner's syndrome may occur (*15,16*). Other symptoms may include dizziness, headache, flushing, palpitations, tachycardia, arrhythmias, diaphoresis and photophobia (*17*).

Physical examination reveals a mass which may be pulsatile, located below the angle of the mandible is typically laterally mobile but vertically fixed. The mass is usually nontender, rubbery, firm and noncompressible. A bruit may be audible. Neurologic abnormalities caused by vagal or hypoglossal nerve involvement and Horner's syndrome are unusual but may appear in some patients.

Based on the size and relationship to the carotid arteries, carotid body tumours may be divided into three groups (*9*) (Table 1).

3.3. *Diagnosis*

Medical history and physical examination are essential for the diagnosis of carotid body tumour. Due to the vascular nature of carotid body tumours, percutaneous needle aspiration or incisional biopsy is contraindicated as it may cause massive haemorrhage, pseudoaneurysm formation and carotid thrombosis (*14,16*). As direct biopsy is not suitable for the diagnosis of carotid body tumour, diagnostic imaging modalities are important in the diagnosis and differential diagnosis of this condition.

Duplex ultrasound is commonly used to evaluate neck masses. Computed tomography (CT) and magnetic resonance (MR) scans, especially MR scans (Figure 1) are better for demonstrating the relationship of the neck mass to adjacent structures (*18,19*). Angiography (Figure 2) is capable of demonstrating unusual blood supply to the carotid body tumour, such as contributions from the internal carotid artery, vertebral artery and thyrocervical trunk and has played an important role for the surgical management of carotid body tumours (*20,21*); although it has been not used very often in some vascular units in recent years (*1*).

3.4. *Treatment*

Surgical resection of a carotid body tumour was first attempted by Reigner in 1880, but the patient did not survive the operation (*22*). Maydl was the first to resect a carotid body tumour on a patient who survived in 1886, although the patient suffered from a postoperative

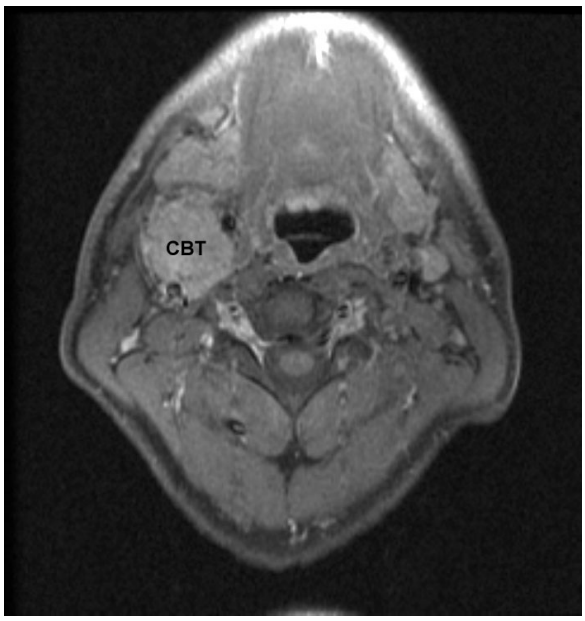

Figure 1. Carotid body tumour (CBT): MRI.

Table 1. Shamblin classification of carotid body tumours

Group	Tumour size	Relationship with carotid arteries	Surgical treatment
Group I	Relatively small	Minimally attach to the carotid arteries	Surgical excision is not difficult
Group II	Larger	Moderately attach to the carotid arteries	Often require a carotid shunt
Group III	Very large	Encase the carotid arteries	Often require arterial resection and grafting

stroke and became aphasic and hemiplegic (*23*). The first successful carotid body tumour excision was performed by Albert in 1889 (*1*). Complete surgical excision of carotid body tumour is still the treatment of choice as the tumour has a 5% or greater incidence of metastases; radiation therapy and chemotherapy are unsatisfactory. Surgical removal of small, asymptomatic carotid body tumour is recommended as it has a much lower risk of cranial nerve and carotid artery injuries than excision of a large tumour. If the size of carotid body tumour is more than 5 cm, operative mortality is 1-3% (*24*).

When the carotid body tumour is large, preoperative embolisation has been used to decrease the vascularity of the tumour and lower operative blood loss thereby reducing technical difficulty (*25-27*). However, preoperative embolisation is still controversial as it may cause internal carotid or cerebral artery thrombosis (*28*).

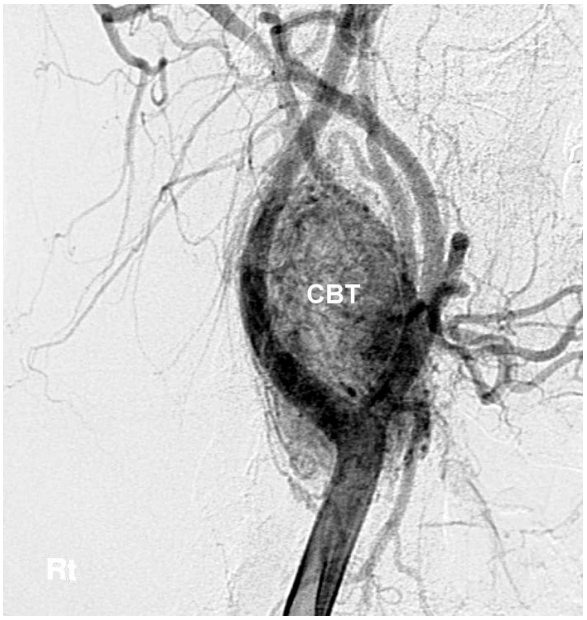

Figure 2. Carotid body tumour (CBT): angiography.

4. Ultrasound assessment of carotid body tumour

4.1. *Ultrasound assessment of carotid body tumours*

Ultrasound scan is a non-invasive, inexpensive and readily available imaging tool, has been used to diagnose carotid body tumours since the late 1970s (*29-32*). The development Doppler colour flow image (*33*) and power Doppler image (*34*) have enhanced capabilities of ultrasound to assess carotid body tumours.

4.2. *Duplex ultrasound features of carotid body tumour*

Duplex ultrasound characteristics of a carotid body tumour is of a highly vascularised solid hypoechoic mass in the area of the carotid bifurcation, which usually causes splaying of the bifurcation and separation of the internal and external carotid arteries (Figures 3 and 4).

On colour flow imaging, hypervascularity of the tumour is seen as irregular colour signals with flow direction being predominantly cephalad (*35,36*). Typical pulsed Doppler flow patterns from carotid body tumour have a low resistance character with a high diastolic component (*37*). Power Doppler imaging shows abundant flow, characterized as intense blush, throughout the tumours (*34*).

4.3. *Role of duplex ultrasound in the diagnosis and assessment of carotid body tumour*

Based on the above described ultrasound features, carotid body tumour is not difficult to diagnose by a duplex ultrasound scan. Since duplex ultrasound is a non-invasive, inexpensive and readily available diagnostic modality, it has been recommended for

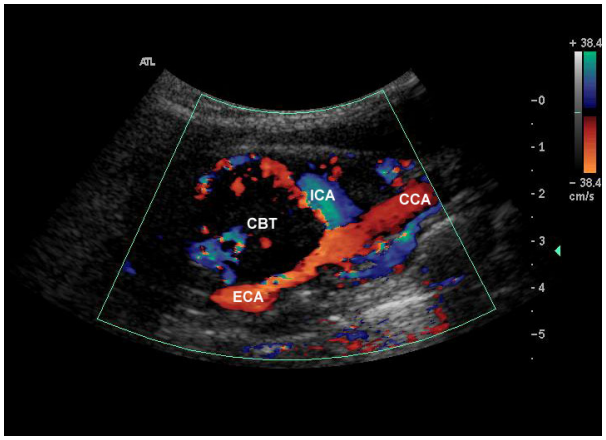

Figure 3. Duplex ultrasound – longitudinal view: carotid body tumour at carotid bifurcation. CCA, common carotid artery; ICA, internal carotid artery; ECA, external carotid artery; CBT, carotid body tumour.

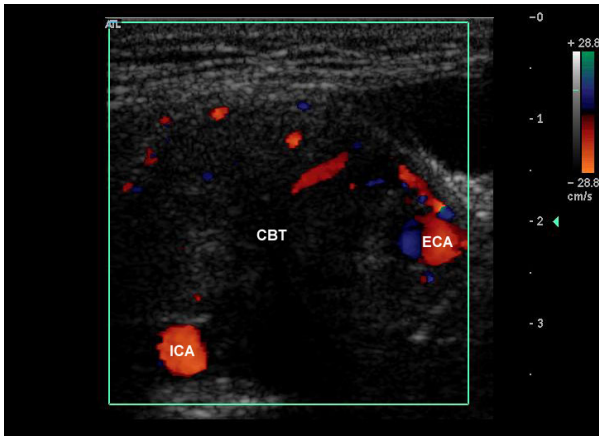

Figure 4. Duplex ultrasound – transverse view: the internal and external carotid arteries are separated by the tumour. ICA, internal carotid artery; ECA, external carotid artery; CBT, carotid body tumour.

screening familial carotid body tumour (*38*).

Vascularity and location are the two key factors used by ultrasound to differentiate carotid body tumours from other neck mass, such as lymphomas, metastatic tumours, thyroid lesions, submandibular salivary gland tumours, and branchial cleft cysts. However, it can be difficult for ultrasound scan to distinguish carotid body tumour from other types of paraganglioma, such as glomus vagale tumour when it is located at the carotid bifurcation, as they have a similar ultrasound appearance (*39*).

In addition to the diagnosis and differential diagnosis of carotid body tumours, duplex ultrasound can provide information on carotid body tumour dimensions, blood supply of the tumour and coexistent carotid artery disease, which is useful in forming a treatment plan and assessing the risk of surgery.

4.4. *Limitations of ultrasound in the assessment of carotid body tumour*

Despite the usefulness of duplex ultrasound in the assessment of carotid body tumour, the limitations of ultrasound include the inability to differentiate carotid body tumour from other type of paraganglioma in the region (*39*) and limited ability to identify complex blood supply to a large carotid body tumour (*33*). CT and/or MRI are better in differentiating of carotid body tumour from other paraganglioma, and angiography is better for the identification of blood supply to the carotid body tumour.

5. Conclusion

Duplex ultrasound is a noninvasive, inexpensive and readily available diagnostic tool, capable of diagnosing carotid body tumour based on its vascularity and location, and can be used as a first-line imaging modality for the diagnosis and assessment of carotid body tumours.

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Commentary

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The classification of acute pancreatitis: Current status

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Summary **The Atlanta Classification of acute pancreatitis (AP) is widely accepted and has been used by physicians and radiologists since 1992. However, advances in knowledge of the disease process, improved imaging, and ever-changing treatment options have rendered some of its definitions ambiguous and highlighted the inadequacy of its classification of severity. This review discusses revision of the Atlanta Classification (2008) and it describes a new determinant-based classification (2012). In contrast to the Atlanta Classification, the revised version and new classification are based on evidence but still need to be developed through systematic review of new data and further international consultation.**

Keywords: Acute pancreatitis, classification, severity

Acute pancreatitis (AP) is an inflammatory disease of the pancreas. It has a mild, self-limiting course in 80% of patients who recover without complications. The remaining patients have a severe disease with local and systemic complications, and this disease carries a mortality risk of 10-24% (*1-3*). The treatment of mild AP is conservative and supportive, but severe episodes may require minimally invasive techniques or even surgical intervention. Thus, the accurate classification of the severity of AP is crucial. Key steps are to define its severity, to monitor the course of the disease, and to make informed clinical decisions. In clinical research, accurate classification of the severity of AP can be used as an effective means of communication among physicians and valid comparison of results from different institutions.

The assessment of AP severity has continually been of interest to clinicians, and several systems to classify pancreatitis emerged in the 20th century (*4-7*). The Atlanta Classification (Table 1) is a clinically based classification system resulting from an international meeting, the 1992 International Symposium on Acute Pancreatitis (*8,9*). Briefly, the Atlanta Classification categorizes AP as "mild" to "severe." The latter is distinguished by organ failure and/or local complications (see the note in Table 1). The Atlanta symposium attempted to offer a global "consensus" and a universally applicable classification system for AP. The definitions of AP, its severity, and organ failure and local complications in the Atlanta Classification are widely accepted and used by physicians and radiologists, representing an important step forward in the classification of AP.

Although the Atlanta Classification has proved useful in the years since 1992, many of its definitions proved confusing and have not been accepted or

Table 1. Summary of the 1992 Atlanta Classification of AP

Severity	Definition
Mild AP	Associated with minimal organ dysfunction and an uneventful recovery; lacks the features of severe AP.
Severe AP	Associated with organ failure[a] and/or local complications[b].

Note:

[a] *Organ failure and systemic complications*
- •Shock: SBP < 90 mmHg.
- •Pulmonary insufficiency: $PaO_2 \leq 60$ mmHg.
- •Renal failure: Creatinine ≥ 170 μmol/L (≥ 2 mg/dL) after rehydration.
- •Gastrointestinal bleeding: 500 mL in 24 hours.
- •Disseminated intravascular coagulation: Platelets ≤ 100, 000/mm^3, fibrinogen < 1.0 g/L and fibrin-split products > 80 μg/L.
- •Severe metabolic disturbances: Calcium ≤ 1.87 mmol/L or ≤ 7.5 mg/dL.

[b] *Local complication*
- •Acute fluid collections.
- •Pancreatic necrosis.
- •Acute pseudocyst.
- •Pancreatic abscess.

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utilized by the pancreatic community. Bollen *et al*. (*10,11*) evaluated the use of the Atlanta definitions in a total of 447 articles, published after 1993, identified by a MEDLINE search. They found that more than half of the studies used alternative definitions of the predicted severity and actual severity of AP and organ failure. Interpretations of the Atlanta definitions of local complications also varied widely.

Increased knowledge of the pathophysiology of necrotizing pancreatitis, improved imaging of the pancreatic parenchyma and peripancreatic collections, and the development of new interventions to manage complications, such as minimally invasive radiologic, endoscopic, and laparoscopic procedures have resulted in several studies identifying shortcomings in the Atlanta Classification. The limitations of Atlanta Classification can be summarized as follows: patients identified as having "severe AP" consist of subgroups with very different outcomes (*12-16*), forms of AP with higher risks of mortality, such as necrotizing pancreatitis (*14,15,17*) (sterile or infected? pancreatic or peripancreatic?), were inadequately described or categorized, and organ failure (*18-22*) was not adequately categorized (transient or persistent?).

In order to establish a more accurate classification system, the Acute Pancreatitis Classification Working Group revised the Atlanta Classification in 2008 (*23*) (Table 2). An obvious feature of the revised classification is that AP is classified into two phases: an early phase (usually within the first week of onset)

Table 2. Revision of The Atlanta Classification of AP

	Severity	Definition
1st week	Non-severe AP	absence of organ failure or the presence of organ failure[a] that does not exceed 48 hours in duration.
	Severe AP	persistence of organ failure that exceeds 48 hours duration (*i.e.*, organ failure recorded at least once during each of three consecutive days).
After 1st week	Interstitial edematous pancreatitis (IEP)	CECT demonstrates diffuse or localized enlargement of the pancreas and normal, homogeneous enhancement of the pancreatic parenchyma.
	Necrotizing pancreatitis[b]	CECT demonstrates the presence of necrosis in either the pancreatic parenchyma or the extra pancreatic tissues. The necrosis should be further classified into as Sterile or Infected.

Note:
[a] Organ failure is defined in accordance with the Marshall scoring system as a score ≥ 2 for at least one of these three organ systems: respiratory; renal, and cardiovascular.
[b] Necrotizing pancreatitis includes the necrosis of the pancreas alone, or the pancreas and peripancreatic tissues, or peripancreatic tissues alone.

Table 3. Determinant-based Classification of AP

	Mild AP	Moderate AP	Severe AP	Critical AP
(Peri)pancreatic necrosis	NO	Sterile	Infected	Infected
	AND	AND/OR	OR	AND
Organ failure	NO	Transient	Persistent	Persistent

Note:
Local Determinant
The local determinant of severity is necrosis of the pancreas and/or peripancreatic tissue. This is covered by the term (peri) pancreatic necrosis.

Definitions
- (Peri) pancreatic necrosis is nonviable tissue located in the pancreas alone, or in the pancreas and peripancreatic tissues, or in peripancreatic tissues alone. It can be solid or semisolid (partially liquefied) and is without a radiologically defined wall.
- Sterile (peri) pancreatic necrosis is the absence of proven infection in necrosis.
- Infected (peri) pancreatic necrosis is defined when at least one of the following is present:
  – Gas bubbles within (peri) pancreatic necrosis on computed tomography
  – A positive culture of (peri) pancreatic necrosis obtained by image-guided fine-needle aspiration
  – A positive culture of (peri) pancreatic necrosis obtained during the first drainage and/or necrosectomy.

Systemic Determinant
The systemic determinant of severity is a certain degree of distant organ dysfunction due to AP. This is covered by the term organ failure.

Definitions
- Organ failure is defined for 3 organ systems (cardiovascular, renal, and respiratory) on the basis of the worst measurement over a 24-hour period. In patients without preexisting organ dysfunction, organ failure is defined as either a score of 2 or more for the assessed organ system according to the SOFA (Sepsis-related Organ Failure Assessment) score or when the relevant threshold is breached, as shown:
  – Cardiovascular: need for inotropic agent
  – Renal: creatinine ≥ 171 μmol/L (≥ 2.0 mg/dL)
  – Respiratory: $PaO_2/FiO_2 \leq 300$ mmHg (40 kPa).
- Persistent organ failure is the evidence of organ failure in the same organ system for 48 hours or longer.
- Transient organ failure is the evidence of organ failure in the same organ system for less than 48 hours.

and a subsequent phase occurring after the first week of onset of the disease. These two phases have a distinct pathophysiology. Because the first phase is characterized more by the presence or absence of organ failure and less by morphologic findings in and around the pancreas, AP should be classified as being in the first phase based on "functional" or "clinical" parameters. In the second phase, the need for treatment is determined by the presence of symptoms and/or complications. Therefore, "morphologic" criteria should be used to classify AP in the second stage because morphologic criteria can be used to guide treatment. Briefly, the clinical classification is used during the early phase of disease (within the first week of onset) while the morphologic classification is used during the subsequent phase (usually after the first week after onset).

Several comprehensive reviews of the available evidence have noted several flaws with this revised classification: *i*) "mild" and "severe" are not sufficient to categorize the severity of AP and cannot differentiate between subgroups with different outcomes (*24-28*); *ii*) the classification of severity should be based on key factors that are causally associated with severity, rather than on descriptions of events that may correlate with severity but are not causally associated with it (*29-31*); *iii*) there are insufficient grounds for ending the first phase 1 week after onset of symptoms. Further, clinical events can occur in individual patients in any order on any day, so severity should be categorized based on key events when they occur and without regard to the sequence they occur in (*32,33*).

Given the aforementioned flaws of the Atlanta Classification, a determinant-based classification of AP severity was developed in 2012 (*34*) (Table 3). Systematic reviews of the evidence and expert opinions have favored this classification over the revised Atlanta Classification. New data and international consultation may lead to a different answer in the future and necessitate further revisions, but the transition from a classification based on "clinical experience" to one based on "evidence-based determinants" is a step in the right direction.

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Letter

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DEBRA International: International cooperation to improve healthcare access for patients with epidermolysis bullosa

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Keywords: Epidermolysis bullosa (EB), patient advocacy organisation, clinical network, therapy development

Epidermolysis bullosa (EB) is a group of genetic conditions causing blistering to the skin and body linings which vary in the extent of symptoms but are always painful and disabling and often life threatening. In its most severe form it is fatal in infancy. Currently there is no cure or effective treatment but good management greatly improves quality of life and current research offers good prospects for therapy development.

DEBRA International is the international organisation coordinating the activities of national EB patient support groups, currently working in over 40 countries, with a strong presence in Europe, North and South America and Australasia and a growing membership in Asia, the Middle East and North Africa. The objectives, both internationally and nationally, are to bring about lasting and effective treatments for all forms of EB as quickly as possible and to provide services to help currently affected individuals and families. DEBRA International recognises that these aims can only be met in partnership with clinicians, researchers, industry, other research funders and governments.

Amongst our current priority areas of work are:

● Funding and facilitating research to develop innovative treatments including gene, cell, protein and small molecule therapies; a number of which are in early stage human clinical trials or where such trials are on the horizon. Normally two calls for research grant applications are made each year and, unusually, there is a single system of international peer review used by all of the DEBRAs funding significant amounts of research. On average, we invest around €3 million each year into new research. An invitation-only research planning conference is held every three years, involving the leading research teams worldwide together with expert patients and industry, to identify opportunities and barriers facing therapy development.

● Identifying potential partners and advisers in industry and venture capital, recognising that expertise in bringing products to market is essential in translating the significant scientific advances being made into available treatments in the clinic.

● The generation of best practice clinical guidelines by groups of specialist clinicians in various areas of importance to people with EB. The guideline on dental care is about to be published and guidelines on cancer management, pain management, nutrition, physical therapies and wound care are in preparation.

● Creating stronger clinical networks of specialist EB centres worldwide to promote sharing of expertise and to facilitate clinical trials. The inaugural meeting of this formal network, EB-CLINET, will be held in Salzburg on 5-7 October 2012.

● The establishment of clinical training opportunities for professionals interested in starting, or improving, a specialist EB clinical service in their own countries including, it is hoped, an online, modular course and mentoring. This programme will be launched at EB-CLINET.

● The creation of an international patient-reported database to understand better the natural history of different forms of EB and the costs of living with the condition. This registry, EBCare, is internet based, *www.EBCare.org*, and we welcome the help of health professionals in encouraging patients to register. It is currently published in English with Spanish and other languages to follow shortly.

DEBRA International welcomes contact and partnership with researchers, health care professionals, industry and anyone interested in our work. For more information, visit *www.debra-international.org*.

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IRDR
Intractable & Rare Diseases Research

Guide for Authors

1. Scope of Articles

Intractable & Rare Diseases Research is an international peer-reviewed journal. Intractable & Rare Diseases Research devotes to publishing the latest and most significant research in intractable and rare diseases. Articles cover all aspects of intractable and rare diseases research such as molecular biology, genetics, clinical diagnosis, prevention and treatment, epidemiology, health economics, health management, medical care system, and social science in order to encourage cooperation and exchange among scientists and clinical researchers.

2. Submission Types

Original Articles should be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables.

Brief Reports definitively documenting either experimental results or informative clinical observations will be considered for publication in this category. Brief Reports are not intended for publication of incomplete or preliminary findings. Brief Reports should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 4 figures and/or tables and 30 references. A Brief Report contains the same sections as an Original Article, but the Results and Discussion sections should be combined.

Reviews should present a full and up-to-date account of recent developments within an area of research. Normally, reviews should not exceed 8,000 words in length (excluding references) and should be limited to a maximum of 100 references. Mini reviews are also accepted.

Policy Forum articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 2,000 words in length (excluding references).

Case Reports should be detailed reports of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient. Case reports may contain a demographic profile of the patient but usually describe an unusual or novel occurrence. Unreported or unusual side effects or adverse interactions involving medications will also be considered. Case Reports should not exceed 3,000 words in length (excluding references).

News articles should report the latest events in health sciences and medical research from around the world. News should not exceed 500 words in length.

Letters should present considered opinions in response to articles published in Intractable & Rare Diseases Research in the last 6 months or issues of general interest. Letters should not exceed 800 words in length and may contain a maximum of 10 references.

3. Editorial Policies

Ethics: Intractable & Rare Diseases Research requires that authors of reports of investigations in humans or animals indicate that those studies were formally approved by a relevant ethics committee or review board.

Conflict of Interest: All authors are required to disclose any actual or potential conflict of interest including financial interests or relationships with other people or organizations that might raise questions of bias in the work reported. If no conflict of interest exists for each author, please state "There is no conflict of interest to disclose".

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4. Manuscript Preparation

Manuscripts should be written in clear, grammatically correct English and submitted as a Microsoft Word file in a single-column format. Manuscripts must be paginated and typed in 12-point Times New Roman font with 24-point line spacing. Please do not embed figures in the text. Abbreviations should be used as little as possible and should be explained at first mention unless the term is a well-known abbreviation (*e.g.* DNA). Single words should not be abbreviated.

Title page: The title page must include 1) the title of the paper (Please note the title should be short, informative, and contain the major key words); 2) full name(s) and affiliation(s) of the author(s), 3) abbreviated names of the author(s), 4) full name, mailing address, telephone/fax numbers, and e-mail address of the corresponding author; and 5) conflicts of interest (if you have an actual or potential conflict of interest to disclose, it must be included as a footnote on the title page of the

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Abstract: A one-paragraph abstract consisting of no more than 250 words must be included. The abstract should briefly state the purpose of the study, methods, main findings, and conclusions. Abbreviations must be kept to a minimum and non-standard abbreviations explained in brackets at first mention. References should be avoided in the abstract. Key words or phrases that do not occur in the title should be included in the Abstract page.

Introduction: The introduction should be a concise statement of the basis for the study and its scientific context.

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Results: The description of the experimental results should be succinct but in sufficient detail to allow the experiments to be analyzed and interpreted by an independent reader. If necessary, subheadings may be used for an orderly presentation. All figures and tables must be referred to in the text.

Discussion: The data should be interpreted concisely without repeating material already presented in the Results section. Speculation is permissible, but it must be well-founded, and discussion of the wider implications of the findings is encouraged. Conclusions derived from the study should be included in this section.

Acknowledgments: All funding sources should be credited in the Acknowledgments section. In addition, people who contributed to the work but who do not meet the criteria for authors should be listed along with their contributions.

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Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223.

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Supplemental data: Supplemental data might be useful for supporting and enhancing your scientific research and Intractable & Rare Diseases Research accepts the submission of these materials which will be only published online alongside the electronic version of your article. Supplemental files (figures, tables, and other text materials) should be prepared according to the above guidelines, numbered in Arabic numerals (*e.g.*, Figure S1, Figure S2, and Table S1, Table S2) and referred to in the text. All figures and tables should have titles and legends. All figure legends, tables and supplemental text materials should be placed at the end of the paper. Please note all of these supplemental data should be provided at the time of initial submission and note that the editors reserve the right to limit the size and length of Supplemental Data.

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