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Laboratory evaluation in rheumatic diseases

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Abstract

Autoantibodies can help clinicians to allow early detection of autoimmune diseases and their clinical manifestations, to determine effective monitoring of prognosis and the treatment response. From this point, they have a high impact in rheumatic disease management. When used

carefully they allow rapid diagnosis and appropriate treatment. However, as they may be present in healthy population they may cause confusion for interpreting the situation. False positive test results may lead to wrong treatment and unnecessary anxiety for patients. Autoantibody positivity alone does not make a diagnosis. Similarly, the absence of autoantibodies alone does not exclude diagnosis. The success of the test is closely related to sensitivity, specificity and likelihood ratios. So, interpretation of these is very important for a proper laboratory evaluation. In conclusion, in spite of the remarkable advances in science and technology, a deeply investigated anamnesis and comprehensive physical examination still continue to be the best diagnostic method. The most correct approach is that clinicians apply laboratory tests to confirm or exclude preliminary diagnosis based on anamnesis and physical examination. This review will discuss these issues.

Key words: Autoantibodies; Rheumatic diseases; Auto-immune diseases; Laboratory biomarkers; Diagnostic markers

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Core tip: Serological and proteomic biomarkers are useful in confirming clinically suspected preliminary diagnosis, monitoring the treatment response and prognosis of autoimmune diseases. Tests for acute phase proteins, rheumatoid factor, anti-citrullinated peptide antibodies and antinuclear antibodies, may support the diagnoses of rheumatic diseases. But these biomarkers should be used beside a careful anamnesis and detailed physical examination. Improper using of these tests may cause false-positive results and unnecessary harmful treatments. The sensitivity, specificity and likelihood ratios of the test must be known. If the test is highly specific, the diagnosis can be confirmed in case of positivity and if it is highly sensitive, the possible diagnosis can be excluded in case of negativity.

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INTRODUCTION

When the organism's own immune system elements attack its own tissue or cells it is called autoimmunity, with the antibodies formed called autoantibodies and the diseases occurring called autoimmune diseases. Autoantibodies can be successfully used to confirm the preliminary diagnosis of autoimmune diseases, to determine prognosis, identify disease activity, and to monitor the response to treatment and medication side effects. From this aspect, they have important roles in the management of rheumatic diseases. When used carefully they allow rapid diagnosis and appropriate treatment. However, in some situations instead of helping the clinician to reach a conclusion, they may cause even more confusion. This is because some positive autoantibodies for many autoimmune diseases may be encountered in healthy population. False positive test results may lead to inappropriate treatment and unnecessary anxiety for patients. Autoantibody positivity alone does not make a diagnosis. Similarly, the absence of autoantibodies alone does not exclude diagnosis. The success of the test is closely related to sensitivity, specificity and likelihood ratios. As a result, in spite of the remarkable advances in science and technology, a deeply investigated anamnesis and comprehensive physical examination still continue to be the best diagnostic method. The most correct approach is that clinicians apply laboratory tests to confirm or exclude preliminary diagnosis based on anamnesis and physical examination. Also common rheumatic diseases like osteoarthritis, rheumatoid arthritis (RA) and psoriatic arthritis (PsA) may be diagnosed without laboratory tests.

In this review we examine serologic and proteomic biomarkers used for diagnosis and monitoring of rheumatologic diseases and common errors in daily practice. This article also reviews the use of inflammatory activity tests currently available in health care.

ACUTE PHASE PROTEINS

One of the characteristic features of rheumatologic diseases is inflammation. The inflammation response developing secondary to tissue damage eliminates pathogens, limits injury and allows tissue regeneration. All of these changes are connected with increases [complement, ceruloplasmin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), ferritin, haptoglobin, fibrinogen, alpha-1 antitrypsin and amyloid A] or decreases (albumin, transferrin, and transthyretin) of some certain proteins. The serum levels of these markers are combined with clinical information and used to assess disease activity and treatment response. However, none of these markers

are unique to a disease. In addition to rheumatic diseases they may increase with infections and malignancy. The most common tests used by clinicians are ESR and CRP.

ESR

The increase in acute phase proteins, especially fibrinogen, occurs with an increase in ESR in plasma concentrations. The protein with the most aggregation effect of all plasma proteins is fibrinogen. This is followed by albumin and globulins^[1]. ESR is observed vertical to gravity in sodium citrate blood after being left for 1 h in Westergren or Wintrobe tubes. ESR is stated in mm (mm/h)^[2]. ESR may increase during the acute phase response to RA, polymyalgia rheumatica (PMR), systemic lupus erythematosus (SLE) and vasculitis. The sensitivity of this test is high; however the specificity is very low. In 10% of RA patients and 20% of PMR patients ESR levels may be within normal limits^[3,4]. It may increase in situations without accompanying inflammation. Additionally errors in the measurement technique (delay in evaluation, tube not held vertical, room temperature) and physiological factors (male sex, age, pregnancy) may cause deviations from the normal levels^[5]. As an expected increase happens in ESR with ageing, it is necessary to make a correction for age. The formula $(age + 10)/2$ is used for women, with the formula $age/2$ for men. For all of these reasons attempting to monitor inflammation with ESR may not work sometimes^[6].

CRP

This name was given due to the ability of the protein to precipitate with pneumococcal C polysaccharide. It is synthesized in the liver during the acute phase response and serum levels may increase up to 1000 times^[7]. The causes to increase ESR also increase CRP. However, the increase and return to normal levels of CRP is more rapid and is not affected by age and sex. It begins to increase within the first 4-6 h after inflammation, peaks at 2-3 d and has a half-life of nearly 18 h^[8]. It has both pro-inflammatory and anti-inflammatory effects^[9,10].

As a general rule, CRP levels are staged as follows: Normal < 0.2 mg/dL, indeterminate = 0.2 mg/dL - 1.0 mg/dL and inflammatory > 1 mg/dL^[2]. While high levels may indicate bacterial infection (> 10 mg/dL), there may be a slight increase observed in situations such as obesity, diabetes, smoking, hypertension, physical inactivity, alcohol, chronic tiredness and depression. Additionally examples of other diseases where CRP is used for diagnosis and monitoring include myocardial infarction and atherosclerosis^[5]. In conclusion CRP, which increases in many inflammatory and non-inflammatory situations, has high sensitivity and lower specificity like ESR.

Rheumatic diseases and acute phase reaction

RA: CRP levels may be used to distinguish RA from osteoarthritis. However in some types of osteoarthritis CRP levels may increase. Due to the previously mentioned properties, CRP is more sensitive compared to ESR in terms of showing variation in disease activity^[11].

Additionally CRP is proportionally better correlated to treatment response and radiologic progression than ESR^[12]. In the early period of the disease, high CRP levels lead to the consideration that a progressive and erosive disease is present and prognosis may be bad. However, CRP levels within normal limits do not mean that there is no disease progression. In 10% of RA cases with active disease, acute phase reaction (APR) levels may be in normal limits^[5]. In clinical practice CRP and ESR are used in scores and indices measuring disease activity.

Ankylosing spondylitis and PsA: Due to increased CRP levels in only 50%-70% of active AS patients, there is no linear correlation between symptoms and disease activity in the APR. The highest CRP levels are measured in patients with peripheral arthritis and uveitis^[13]. However, there is no correlation between severity of enthesitis and ESR^[14]. The BASDAI score is slightly better correlated to CRP values compared to ESR^[15]. For evaluation of treatment response, the sensitivity and specificity of CRP and ESR are low. As a result to increase efficiency it is recommended to use both tests together^[13,16].

Some composite measures, such as BASDAI have had limitations for the measurement of disease activity because it is a subjective measure with fully patient oriented and have lacked validity. Thus the Assessment of Spondylo Arthritis International Society proposed to use CRP which is an objective determinant of inflammation and developed ASDAS with higher construct validity^[17]. This was the first to combine patient reported and objective parameters to understand the severity of disease activity.

PMR: This disease characteristically has high ESR and CRP levels. They have very good negative predictive values. And in EULAR/ACR 2012 provisional classification criteria they have been proposed as diagnostic parameters^[18]. However, up to 20% of patients may have ESR at normal levels^[19]. There is a very strong correlation between ESR-CRP and corticotherapy response. However, it should not be forgotten that steroid dose should be regulated according to the patient's clinical symptoms and not ESR and CRP levels^[2]. The steroid use has been detailed in EULAR/ACR 2015 recommendations^[20].

SLE: In spite of active disease and increased ESR, CRP levels are frequently normal or slightly increased^[21]. Increased ESR values may be the first indicator of disease. CRP increases in the presence of severe infection, synovitis and serositis. Slightly high CRP may be a precursor of atherosclerosis^[9,22].

AUTOANTIBODIES

Rheumatoid factor

Rheumatoid factor (RF) is a specific antibody formed against Fc section of immunoglobulins. Though every class of these antibodies have Ig structure, the most common is IgM structure^[23]. The role of RF in RA is not fully known.

However, it may play a role in antigen presentation and amplification of the humoral response^[2]. In nearly 70% of RA patients it is positive and may be an indicator of worse prognosis. High RF levels may show aggressive joint disease, rheumatoid nodules and accompanying extra-articular involvement^[24]. RF positivity alone is not sufficient for diagnosis. In the healthy population 15% may be positive at low titrations and this rate increases with age^[25]. Additionally in other autoimmune rheumatologic diseases including Sjogren's syndrome, SLE, cryoglobulinemia, pulmonary diseases such as interstitial fibrosis and silicosis and various infectious diseases, RF may be positive^[25,26]. Nearly 30% of RA patients are seronegative and this rate may increase to 50% in early RA^[27]. As a result, negative RA may not exclude diagnosis. Due to contradictory results, it cannot be used for monitoring treatment response and disease^[28]. Due to all of these reasons, only in patients where RA is a strong possibility after anamnesis and physical examination should RF be requested.

Anti-citrullinated peptide antibodies

In a large proportion of RA patients IgG antibodies developed against citrulline peptides are encountered. Many studies have determined that the target of these antibodies is a type of protein, filaggrin. These antibodies are post translationally altered or target citrullinated filaggrin. The posttranslational citrullination procedure includes deiminization of arginine in certain polypeptides and is catalyzed by the peptidylarginine deiminase (PAD) enzyme. The result of this biochemical process is that arginines transform to citrullines. These changes in the structure of citrullinated peptides make them a target for the IgG antibodies in RA^[29]. The pioneer of these antibodies identified in 1964 was anti-perinuclear factor. In the intervening period many different antibodies have been described and all of these are given the collective common name anti-citrullinated peptide antibodies (ACPAs). Anti-perinuclear factor, anti-keratin antibody, anti-filaggrin, anti-Sa and anti-cyclic citrullinated peptide (anti-CCP) are the primary members of this family^[30]. As anti-CCP has higher specificity compared to RF, it is more commonly used for RA diagnosis and has taken its place in new classification criteria^[31]. The first generation anti-CCP test (anti-CCP1) had 96% specificity and 53% sensitivity for RA. The second generation anti-CCP test (anti-CCP2) had specificity of 99% and sensitivity of 61.6% for early RA, 75.2% for late RA and 71.7% for all RA patients^[30]. Thus a test with similar sensitivity as RF but with higher specificity was obtained^[32].

Anti-CCP antibodies occur years before the development of clinical symptoms and RA patients are divided into two groups as ACPA positive and ACPA negative^[33,34]. In the early stages of disease the groups show similar characteristics, but with time the ACPA positive group are observed to have more erosion and the disease progresses more severely^[35]. Some environmental factors, especially smoking, increase the risk of ACPA development. ACPA positivity increases the risk of cardiac disease^[36,37]. In a study, researchers found ACPA-mediated activation of platelets. They have suggested that ACPA-mediated

platelet activation may lead to increased vascular permeability and erosive damage^[38,39].

Anti-CCP test should be requested for patients clinically suspected of RA. If it is positive once, there is no need for repeat because anti-CCP antibody titrations are not correlated with disease activity. As a result, it cannot be used to monitor the disease^[40].

ANTIBODIES TO NUCLEAR ANTIGENS

Antibodies generally developing against DNA, RNA, histones, centromeres, nucleolus and other nucleoproteins in the cell nucleus, sometimes targeting organelles, other cytoplasmic structures and even cell membrane are called anti-nuclear antibodies. Clinically the most commonly used antigens are DNA and RNA protein complexes^[41].

When these antibodies are identified in blood they may indicate an emerging rheumatic disease, they may be determinants to make diagnosis and may provide important information related to prognosis.

There has been a clear change in antibodies to nuclear antigen (ANA) measurement techniques since lupus erythematosus (LE) cell was identified in 1940 to the present day when immunofluorescent (IF) techniques are used. Together with the variation in laboratory methods, the performance of the ANA test has changed. With an increase in sensitivity of the test, the probability of observing "ANA-negative lupus" has decreased; however the ANA positivity in healthy individuals has increased. As a result the cut/off value for the test has increased from 1/40 to 1/80^[42].

ANA may be measured in two ways. The first ANA measurement assesses all generic antibodies and is a specific antibody assay that may be specific for other diseases^[43]. Generic ANA measurement may be completed with IF and ELISA methods. If ANA is positive, specific antibodies may be researched with automated methods. IF is the gold standard for ANA identification. For those with clinical suspicion it is significant if identified at high titrations. A study conducted on healthy people found that at 1/40 dilution 31.7% were ANA positive, while this value was 13.3% for 1/80 dilution, 5.0% for 1/160 dilution and 3.3% for 1/320 dilution^[44]. As a result, high titrations are clinically more significant. However, at high titrations correlation with disease activity and severity is not possible^[41]. So it is not correct to attempt to monitor disease activity with ANA values^[2].

ANA staining patterns may provide an idea of specific disease by showing which specific antibodies entered a reaction with which region of the cell. These patterns are usually reported as either nuclear, centromere, or nucleolar. Homogenous, speckled, peripheral, and nucleolar staining patterns are more frequently encountered and have clinically important meanings. This is detailed in Figure 1^[45]. However, it should not be forgotten that reporting of these staining patterns is closely related to the experience and competence of laboratory staff. To avoid this operator-dependent situation, automated tests have received attention and have been commonly used. These

techniques are immunodiffusion, immunoprecipitation, radioimmunoassay, hemagglutination, enzyme immunoassay and enzyme-linked immunosorbent assay^[2]. American College of Rheumatology points IF ANA as the gold standard for ANA testing because it still has more sensitivity than solid phase assays. Laboratories must indicate ANA testing method in their reports^[46].

We know that two major types of antibodies exist in ANA, one including antibodies against DNA and histones which indicates SLE and drug-induced lupus erythematosus (DILE). The second group includes autoantibodies to extractable nuclear antigens. This group contains autoantibodies to Smith antigen (Sm) ribonucleoproteins (RNP), Ro/SSA or La/SSB, Scl-70, histidyl-tRNA synthetase (Jo-1), and PM1. Centromere protein (CENP)-B, topoisomerase- I (topo- I), RNA polymerase I -III (RNA-pol I -III), TM, MU, Mi-2, Ku and RA33 are also in this group and the number of new indicators are increasing day by day^[45].

Interpretation of ANA test

Basic statistics: The sensitivity of a test is the proportion of affected individuals with a positive test and the specificity is the proportion of unaffected individuals with a negative test. Tests with highest sensitivity or specificity have much potential to make differential diagnosis. If a test is highly specific, then positive results points the diagnosis in a high probability. Negative reports of a highly sensitive test can almost exclude the diagnosis.

The likelihood ratio (LR) is one of the efficient ways to reach diagnostic accuracy taking using both sensitivity and specificity. A positive test with a positive LR for any disease indicates the multiplied probability of the diagnosis. A negative test with a negative LR for a disease shows the odds of the decreasing probability^[47]. Taking a detailed history and performance of a careful physical examination is very important to get the pretest probability of a RD. Then using this value, we can get the post test probability of a RD by processing the LR of a test by the help of LR nomogram (Figure 2)^[46].

An ANA test is not a routine test which is requested for any patient with a musculoskeletal symptom and must be used only if we suspect the existence of a RD. ANA test has a sensitivity of 93% for SLE and 85% for scleroderma. On the other hand specificity of ANA for the same diseases are much lower than sensitivity rates (SLE: 57%, scleroderma: 54%). So ANA negativity is an indicative finding to exclude SLE, however its positivity seems not to be so important to as the specificity is relatively lower. Similarly a negative ANA is more meaningful to rule out scleroderma while a positive report do not confirm diagnosis exactly although it supports^[2,42].

For drug-induced SLE and mixed connective tissue disease (MCTD) ANA is a diagnostic criteria as the sensitivity is almost 100%^[42]. The diseases with lower rates of ANA sensitivity are secondary Raynaud's syndrome (64%), polymyositis/dermatomyositis (61%) and Sjögren's syndrome (SS) (48%)^[2,48,49]. ANA is useful in SS and idiopathic inflammatory myositis despite its relatively lower sensitivity for these diseases (40% and 70%). ANA



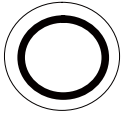

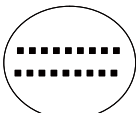
ANA pattern	Antigen	Associated diseases
Speckled 	ENA, RNP, Sm, Ro/SSA, La/SSB, Scl-70, Jo-1, ribosomal-P	SLE, MCTD, systemic sclerosis, Sjögren's syndrome, PM
Homogenous 	dsDNA, Histones SLE	Drug-induced SLE
Peripheral (rim) 	RNP, Sm, Ro/SSA SLE	Systemic sclerosis
Nucleolar 	Anti-PM-Scl, anti-RNA polymerase I -III, anti-U3-RNP, To RNP	Systemic sclerosis, PM
Centromere 	CENP A-E	Limited systemic sclerosis

Figure 1 Common immunofluorescence antinuclear antibodies patterns associated with specific diseases^[45]. ENA: Extractable nuclear antigens; RNP: Ribonucleoproteins; SLE: Systemic lupus erythematosus; MCTD: Mixed connective tissue disease; PM: Polymyositis; dsDNA: Double-stranded deoxyribonucleic acid; CENP: Centromere protein.

is even worse in case of specificity with lower values^[42].

For the diseases generally indicated by specific antibodies, contrary to generic ANA, specificity is more meaningful as they are extremely high unlike their sensitivity values. The most important of these antibodies are:

Anti-dsDNA antibodies: It is the diagnostic criteria of SLE (97.4% Specificity and 57.3% sensitivity, +LR: 16 and -LR: 0.49)^[2,45].

Anti-Sm antibodies: Anti-Sm antibodies reveals mostly and only in SLE patients (sensitivity: 25%-30% and specificity: Very high)^[2].

Anti-RNP antibodies: They can be shown in 30%-60% of SLE patients, however not specific enough. They have use in the diagnosis of MCTD. Anti-U1 RNP antibody is among the diagnostic criteria of MCTD^[2].

Anti-histone antibodies: They are present in 95% of

DILE patients and 50%-70% of those with SLE. A lot of patients revealing the antibodies are asymptomatic so, the positive sera does not always mean the disease exists^[2].

Anti-chromatin (anti-nucleosome) antibody: Present in 50%-90% of SLE patients^[50].

Anti Ro/SSA - anti La/SSB antibodies: They are often shown in SS and SLE patients and also are among the diagnostic criterion of SS^[51]. And these antibodies may be encountered in SLE patients with negative ANA^[2].

Anti-centromere antibodies: Three major centromere proteins exist: CENP-A, B, and C. The major target is CENP-B^[52]. They have relation with limited cutaneous systemic sclerosis and the CREST syndrome^[53]. The specificity in CREST syndrome is high, while sensitivity is lower. Anti-centromere antibodies can estimate the upcoming development of scleroderma in patients with Raynaud's syndrome (+LR: 3.5). However, they are

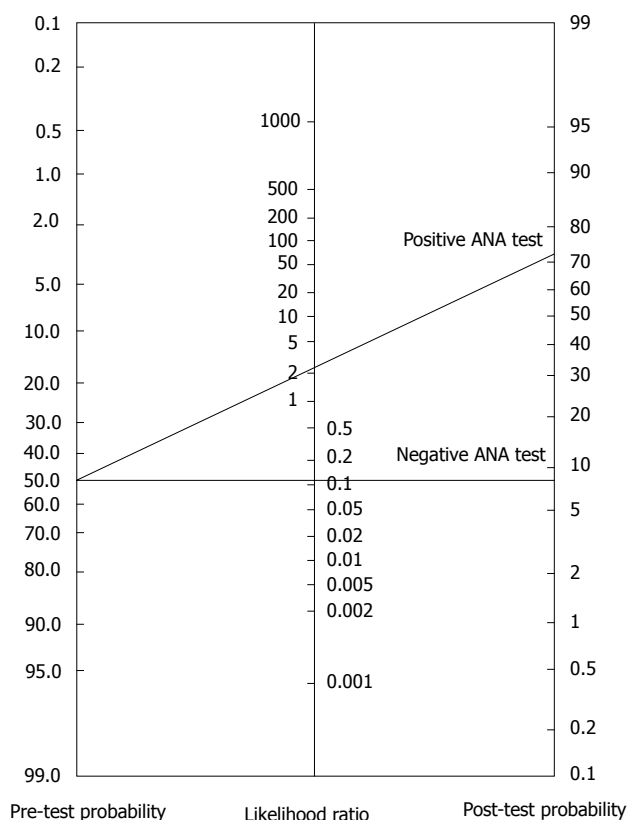


Figure 2 The likelihood nomogram used in systemic lupus erythematosus with an antinuclear antibody test.

more discriminative for excluding CREST (-LR: 0.2).

Anti-Scl-70 antibodies: They are found in approximately 20%-40% of patients with systemic sclerosis. Their presence predicts pulmonary fibrosis, diffuse cutaneous involvement, and nephropathy. Although the sensitivity is low, specificity approaches 100%. It shown in patients with Raynaud's syndrome, the diagnosis of scleroderma is highly probable as specificity is 98% and positive LR is 10. On the other hand the sensitivity is low (28%, negative LR is 0.7)^[2].

Anti-nucleolar antibodies: The nucleolar IF pattern is very specific for scleroderma. Specific antibodies which form this pattern are anti-PM/Scl antibodies, anti-Th/To antibodies, anti-RNA polymerase I, anti-RNA polymerase III and anti-U3-RNP^[54].

Other antibodies: The presence of anti-neutrophil cytoplasmic antibodies (ANCA) is supportive in the diagnosis of vasculitic conditions. These antibodies demonstrate two forms of IF patterns: Cytoplasmic (cANCA) and perinuclear (pANCA). The cANCA has a high sensitivity and a low specificity (90%, 50% respectively) in Wegener's granulomatosis^[2]. The pANCA form is shown frequently in pauci-immune glomerulonephritis, microscopic polyangiitis, Churg-Strauss syndrome, and sometimes in Wegener's granulomatosis^[47,55].

The myositis-specific antibodies are not often used

for the identification of inflammatory myopathies; but, they can provide evidence about the manifestations of the disease once the diagnosis is made^[2]. In 25%-30% of the patients with dermatomyositis or polymyositis the Jo-1 ANA can be detected^[56]. Anti-Mi2 antibodies are also seen in dermatomyositis and are a predictor of good prognosis. Anti-SRP is related with heart disease and is responsiveness to treatment. Anti-MAS is identified in rhabdomyolysis^[2].

CONCLUSION

In conclusion, laboratory tests are useful for informing us for an emerging RD. They help diagnose a specific disease and can predict prognosis. An experienced clinician must first evaluate the patient with clinical approaches and then request meaningful laboratory tests as complementary diagnostic tools. Interpretation of laboratory tests necessitates to know the diagnostic power of each test.

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Chromogranin A as a valid marker in oncology: Clinical application or false hopes?

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Abstract

Chromogranin A, due to its primary expression throughout the neuroendocrine system, is a widely accepted biomarker for the assessment of neuro-endocrine tumors. It has been traditionally used in the management of patients with tumors of gastro-enteropancreatic origin. Lately, it has also been implicated in various conditions and diseases, both benign and malignant. However, the paucity of data of adequate strength, as well as its relation with common physiologic conditions and its interaction with commonly prescribed medications, limit its clinical use in only a narrow spectrum. Herein, we present a thorough review to the most frequent conditions where its levels are affected, focusing specifically on its potential use as a prognostic and predictive biomarker in oncology.

Key words: Cancer; Neuroendocrine tumors; Prognosis; Chromogranin A; Biomarker

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Core tip: In the era of targeted therapy, there is an unmet need for the development of more sensitive, specific and reliable biomarkers for early diagnosis, prognosis and detection of early recurrence to tumors which comprise an extremely heterogeneous group.

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INTRODUCTION

The Granins comprise a family of proteins whose most well known members are chromogranin A (CgA), chromogranin B (CgB) and secretogranin II, with their most common characteristic being their acidic profile. They are produced as pre-proteins in the ribosomes and subsequently they undergo post-translational modifications in the endoplasmic reticulum and in the Golgi apparatus^[1]. It has been shown that they are co-stored with peptides and amines in the granules of endocrine cells. They can also be found in a number of other cells, including immune cells, epithelial cells and peripheral neurons^[2]. Other proteins that are also included in the granin family are secretogranin III, the HSL-19 antigen (secretogranin IV), the neuroendocrine secretory protein 7B2 (secretogranin V), NESP55 (secretogranin VI) and nerve growth factor-inducible protein VGF (secretogranin VII)^[3].

Granins are composed of single-polypeptide chains of approximately 180 to 700 amino acids, with CgA being a 49 kDa protein produced mainly by endocrine and neuroendocrine cells^[1,4,5]. It was first discovered in the chromaffin granules of the adrenal medulla, where it is stored along with the resident hormones, like calcitonin, and then secreted with them^[5]. The CgA gene, located on chromosome 14, is probably a single copy gene rather than a member of a dispersed, multigene family^[6].

Since the discovery of CgA and its pathologically high levels in patients with neuroendocrine tumors, it has been correlated with a number of other conditions, both benign and malignant (Tables 1 and 2). Its sensitivity and specificity in each one of these conditions differ significantly, depending on various factors, limiting its use as an effective prognostic and/or predictive marker in a narrow spectrum of conditions. This review summarizes the most frequent conditions where CgA levels are affected, focusing specifically on its function as a biomarker in oncology.

CgA may be secreted in the blood in its full length or in fragments after cleavage. These fragment peptides include Catestatin, Chromacin, Pancreastatin, Parastatin, Vasostatin I, Vasostatin II and WE-14^[1]. Although CgA and its peptides definite functions have not been fully understood, it is believed that they are important factors for the formation and regulation of dense-core granules, heart function, catecholamines and parathyroid hormone secretion, carbohydrate and lipid metabolism, immune properties and reproduction^[7].

CGA IN NON-MALIGNANT DISEASES AND CONDITIONS

CgA has been correlated with a wide range of non-malignant systemic diseases, including hypertension, heart and hepatic failure (Table 1)^[1,8]. It is produced by the human myocardium and exerts negative inotropic effect, so in chronic heart failure it is significantly elevated and its levels can parallel the severity of cardiac dysfunction and

could be used as an independent predictor of mortality^[8]. Furthermore, basal plasma CgA levels correlate with sympathetic tone and increased adrenal sympathetic nerve activity. Subsequently, CgA levels are usually elevated in hypertension^[8].

Furthermore, it can be raised in renal insufficiency, as a result of decreased plasma clearance. It has also been implicated in inflammatory and autoimmune conditions, like Rheumatoid arthritis^[9,10]. Furthermore, PPIs, which are some of the most commonly prescribed drugs, may cause a secondary increase in CgA levels due to increased gastrin production^[11]. Another common condition that is associated with elevated levels of CgA, is chronic atrophic gastritis (Table 1)^[12]. Summarizing, in non malignant diseases and conditions, CgA values may reach values of hundreds (ng/mL), but it is very uncommon to reach levels of several thousands that could be consistent with cancer diagnosis.

CGA IN MALIGNANT DISEASES

Bronchopulmonary neuroendocrine tumors

In small cell lung carcinomas (SCLC) the mean CgA plasma levels are higher than those found in normal controls or in patients with chronic obstructive pulmonary disease, lung adenocarcinoma and large-cell lung carcinoma. The levels of CgA are associated with the extent of the disease, but the levels of NSE have been proven to be more accurate in that regard^[13-16]. Bronchopulmonary neuroendocrine tumors (BP-NETs) comprise approximately 20% of all lung cancers and represent a spectrum of tumors arising from neuroendocrine cells of the BP-epithelium. Although they share structural, morphological, immunohistochemical, and ultrastructural features, they are separated into 4 subgroups: Typical carcinoid tumour (TC), atypical carcinoid tumour (AC), large-cell neuroendocrine carcinoma (LC-NEC), and SCLC^[17]. The diagnosis is based on the recognition of neuroendocrine morphology, such as organoid pattern, and on the immunohistochemical demonstration of specific neuroendocrine markers, like chromogranin, synaptophysin, and neural cell adhesion molecule (NCAM), also known as CD 56. To confirm the neuroendocrine origin of the tumour cells, at least one of those markers must be positive^[18]. Although they can produce a variety of peptides and hormones, like gastrin-releasing peptide (bombesin) and 5-hydroxytryptophan, bronchial NETs only occasionally secrete bioactive products that can easily be measured. As a result, elevated plasma or urinary hormone levels are only rarely detected. Serum levels of CgA are lower in bronchial NETs than those observed in NETs of other sites, and they overlap with those seen in patients who have non-malignant conditions associated with increased CgA levels^[17].

Breast cancer

In breast cancer CgA was discovered both in epithelial cells of normal mammary gland as well as in breast cancer. However, it does not seem to offer any additional

Table 1 Non cancerous causes of chromogranin A elevation

Disease			
Cardiovascular	Endocrine	Gastrointestinal	Inflammatory
Acute coronary syndrome	Hyperparathyroidism	Chronic atrophic gastritis	Chronic bronchitis
Arterial hypertension	Hyperthyroidism	Chronic hepatitis	Chronic obstructive pulmonary disease
Cardiac insufficiency	Hypercortisolism	Inflammatory/irritable bowel syndrome	Giant cell arthritis
		Liver cirrhosis	Rheumatoid arthritis
		Pancreatitis	Systemic inflammatory response syndrome
Drugs			
Corticoids	H2 receptor antagonist	Proton pump inhibitor	
Status			
Exercise	Ingestion of a meal	Pregnancy	
Factors having potential influence on sample			
Fibrin presence	Haemolysis	Imposing effect: Autoantibodies presence (RF-IgM, Avidine, Heterofile)	Late afternoon/night > morning
Lipaemia	Plasma > serum	-	

Table 2 Frequent cancer-related causes of increased chromogranin A

Cancer	Neuroendocrine tumors
Breast	Colorectal
Colon	Gastric
Hepatocellular	Medullary thyroid
Ovarian	Neuroblastoma
Pancreatic	Pancreatic
Prostate	Paraganglioma
	Pheochromocytoma
	Pituitary
	Small cell lung
	Small intestinal

information about the presence, the extent and the histology of breast cancer when compared to the more established Ca 15-3. Furthermore, serum CgA was not sensitive enough to identify the rarely encountered subtype of breast cancer with neuroendocrine differentiation^[19].

Merkel cell carcinoma

Merkel cell carcinoma (MCC) is a rare, aggressive, cutaneous malignancy with neuroendocrine differentiation, that predominantly affects older adults with light skin complexion. MCC has a propensity for local recurrence and regional lymph node metastases. On immunohistochemistry, the tumour cells show features of both epithelial and neuroendocrine origin, including the expression of CgA. CgA blood levels are used by many physicians as a predictive marker for the response of the tumour to chemotherapy, though it has never been shown to correlate with progression-free survival, disease specific survival, or disease recurrence^[20].

Gastroenteropancreatic neuroendocrine tumors

Chromogranins were early discovered to be elevated in the plasma of patients with neuroendocrine tumors^[21,22]. They arise from neuroendocrine cells that occur throughout the length of the entire gut, and about two-thirds of them are of gastrointestinal or pancreatic origin (GEP-NETs)^[23]. Their relevance in the diagnosis, prognosis, clinical evaluation

after cytoreductive surgery, and subsequent follow-up of patients with those types of tumors, has been studied for more than 20 years^[21].

Although GEP-NETs excrete a number of peptides specific to the neuroendocrine cell of origin, CgA is the most frequently studied biomarker for their diagnosis and subsequent follow-up^[24-26]. Not all GEP-NETs produce CgA, but for those that do, elevated circulating levels of CgA could be related with tumour burden as well as recurrence, and are considered a marker of poor prognosis and reduced survival in both ileal and pancreatic NETs^[27,28]. For example, in patients with midgut carcinoids the 5-year OS was estimated to be 22% with CgA levels > 75 nmol/L, while it was raised to 63% with levels lower than this value. The decrease of CgA levels has also been used as a marker of response to treatment in clinical trials, where biochemical response is defined as a $\geq 50\%$ reduction of CgA^[29].

The highest levels of CgA are observed in patients with functioning ileal NET and carcinoid syndrome, followed by those with liver metastases. Metastatic disease in the lymph nodes does not seem to cause a significant increase in the levels of CgA^[27,29]. However, its value in predicting liver metastases, as compared to morphological tumour changes as measured by CT or MRI, is limited, with a sensitivity and specificity of 71% and 50% respectively^[30]. On the contrary, it should be noted that its elevation, even in values of several thousands (ng/mL), could be not related with deterioration of clinical status.

The overall sensitivity of CgA in the diagnosis of neuroendocrine tumors is around 60%-80% and depends on the primary site, on the degree of differentiation and on the status of the disease^[31]. This marker has a low sensitivity regarding its use in distinguishing the different types of NETs. It should be noted also, that the specificity and sensitivity of the assay for CgA measurement differ between the available commercial kits^[32].

Moreover, the use of CgA as a diagnostic biomarker in GEP-NETs has certain limitations. Firstly, although CgA could be useful in predicting tumor relapse or progression, with rapidly increasing levels correlating

Table 3 Chromogranin A diagnostic accuracy in neuroendocrine tumor studies

Type (no pts)	CgA cut-off	Sensitivity (%)	Specificity (%)	Ref.
NET (128)	100 µg/L	59	68	[57]
NET (127)	34.7 u/L	67.9	85.7	[35]
NET (80)	17 u/L	56.3	100	[58]
NET (63)	34 u/L	55	94	[59]
GEP/NET (61)	20 u/L	92	83	[50]
	100 u/L	47	99	
GEP/NET (124)	130 µg/L	62.9	98.4	[16]
GEP/NET (202)	53 ng/mL	71.3	77.8	[60]
NET (120)	98 ng/mL	79	NA	[61]
GEP/NET (119)	2.8 nmol/L	92.9	100	[62]

no: Number; pts: Patients; NA: Non available; CgA: Chromogranin A; NET: Neuroendocrine tumor; GEP: Gastroenteropancreatic.

with shorter survival, it should be noted that CgA levels are also affected by the secretory activity of a functioning tumor. This has particular importance in patients treated with somatostatin analogues (SSAs), where the drop in CgA levels may reflect the inhibition of the secretory activity of the tumour rather than a true anti-tumour effect^[33].

Midgut carcinoids have often been misdiagnosed as irritable bowel syndrome or inflammatory bowel disease, where CgA may also be increased, due to the common manifestation of watery diarrheas^[34].

CgA along with NSE have been retrospectively studied as prognostic biomarkers in GEP-NETs^[35]. In a phase II study of Everolimus in GEP-NETs it has been demonstrated that higher baseline levels of CgA were associated with shorter PFS, while the patients with the shortest PFS had elevated concentrations of both CgA and NSE at baseline. In that same study, CgA and NSE responses were defined as a 50% or greater reduction from baseline or normalization, and early CgA and NSE responses were defined as a 30% or greater decrease from baseline or normalization after 4 wk of treatment. For both those markers, an early decrease predicted for clinical benefit, which, in the case of CgA, meant both longer PFS (13.3 mo vs 7.5 mo; HR = 0.25; $P < 0.001$) and longer OS (24.9 mo vs 12.7 mo; HR = 0.4; $P = 0.01$)^[36].

Those results have been confirmed in a relevant analysis of the phase III RADIANT-2 clinical trial, where it was shown that early decrease of CgA levels by Everolimus can be used as a surrogate marker of PFS in this setting^[37]. To our knowledge, no such data exist for patients with GEP-NETs treated with Sunitinib.

There is no doubt that due to the existing data, CgA role in NET diagnosis is strongly limited and debated. Therefore, it could not be recommended and applied in our daily clinical practice. Moreover, it could be used primarily but with caution, in NETs as a marker of therapy response.

Prostate cancer

CgA is excreted by the neuroendocrine cells that are

dispersed throughout the prostatic gland. Neuroendocrine cells can be found in the normal prostate as well as in benign prostate hyperplasia and in primary or metastatic prostatic adenocarcinoma^[38]. In addition to CgA, neuroendocrine cells produce a variety of biogenic amines, such as NSE, calcitonin and somatostatin. According to their degree of differentiation, prostatic malignant neuroendocrine cells may continue to produce those amines, though they differ in their morphology from their normal counterparts^[39].

Although not specific for prostate cancer, there is evidence that high levels of serum CgA are a marker of advanced disease, associated both with high tumor grade and later stage^[40]. High levels also characterize the shift from a disease responding to androgen deprivation therapies (ADT) to an androgen-independent, aggressive malignancy^[41,42]. Pathophysiologically, this is to be expected, since an increase in circulating CgA and NSE reflect tissue neuroendocrine differentiation. There is evidence that the degree of neuroendocrine differentiation increases with prostate cancer progression, and it has been suggested that it constitutes a major mechanism of resistance to ADT^[38]. Neuroendocrine cells do not express androgen receptors, consequently they are not regulated by androgens^[43].

There is also evidence that serum CgA, either alone or combined with serum PSA, may predict poor prognosis in castration-resistant prostate cancer following endocrine therapy^[44-46]. Moreover, circulating neuroendocrine peptides have been linked with angiogenesis and invasive potential^[39,47]. However, serum concentration of CgA and tissue IHC expression do not show robust correlation and CgA does not seem to positively correlate with treatment response to cytotoxic chemotherapy in metastatic prostate cancer with neuroendocrine differentiation^[48].

Multiple Endocrine Neoplasia type 1 syndrome

Multiple Endocrine Neoplasia type 1 (MEN 1) is a rare hereditary autosomal dominant endocrine cancer syndrome, that is characterized by the development of tumors, both benign and malignant, in multiple endocrine organs. The tumors most often appear in the parathyroid glands, in the endocrine cells dispersed throughout the gastroenteropancreatic (GEP) tract and in the anterior pituitary, though other endocrine and non-endocrine tumors have also been reported, namely adrenocortical and thyroid tumors, visceral and cutaneous lipomas, meningiomas, facial angiofibromas and collagenomas, and thymic, gastric, and bronchial carcinoids^[49].

Several studies have assessed the role of CgA in demonstrating the presence of a GEP-NET in MEN 1 syndrome. It has been confirmed that abnormally elevated CgA levels are highly suggestive of both sporadic and MEN 1-related GEP-NETs. The highest levels are observed in metastatic disease, especially when the metastases are located in the liver, and in functioning tumors, especially in gastrinomas^[50]. In MEN 1 patients without biochemical or imaging evidence of GEP tumors, the data are scanty and conflicting. Some studies have reported increased CgA

levels in 11%-33% of patients with pituitary adenomas, both secreting and non-functioning^[51]. In addition, conflicting data have been published regarding the relationship between CgA levels and hyperparathyroidism, either primary or in the context of MEN 1 syndrome^[52]. However, it appears that the generalised hyperplasia of the endocrine system, that occurs in MEN 1 syndrome, tends to lead to at least mildly elevated levels of circulating CgA, while markedly raised levels may indicate the presence of a GEP-NET^[50].

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) represents the most frequent complication and a major cause of death in patients with cirrhosis of any aetiology^[53]. The most widely used biomarker for diagnosis and follow-up is AFP^[54]. CgA has been found elevated in patients with liver cirrhosis and in those with HCC^[55,56]. However, its use as a diagnostic biomarker for the presence of HCC in the context of cirrhosis should be discouraged, since the levels of CgA have not been found to differ significantly between these two conditions^[54]. The prognostic meaning of CgA in HCC has yet to be elucidated.

DISCUSSION

The extent of the physiological functions of CgA indicates its potential role as a biomarker in a wide spectrum of benign and malignant diseases (Tables 1 and 2). However, certain factors limit its usefulness in only a few. There is a lack of prospective studies that aim to evaluate its validity in the diagnosis and prognosis of specific conditions.

Although limitations exist, CgA is the most studied biomarker for GEP-NETs' diagnosis and management. Clinicians should be aware of the variation of measurements by numerous physiologic and pathologic conditions, its limited predictive value and the modest sensitivity (Table 3)^[57-62]. Moreover, data support that baseline CgA levels and changes during treatment are prognostic. Even, its specificity could be heavily affected by several benign conditions, also intrinsic features of the disease could be related with the high variability of CgA values^[63]. Diagnostic accuracy of CgA for GEP-NETs appear to be higher for well vs poorly differentiated tumors, functioning vs non-functioning, metastatic vs locoregional disease. There is no doubt that it is more reliable when used to evaluate response to therapy or disease progression than early diagnosis or recurrence.

It should be underlined that there are many assays and commercial kits available for CgA levels evaluation, thus very strict quality assurance and standardization should be used. In addition, CgA evaluation is more convenient than U5-HIAA, which requires a 24-h urine collection and 3 d before the collection a dietary abstinence from tryptophan/serotonin-rich foods.

Finally, in cancers where a biomarker is already in use, such as AFP in hepatocellular carcinoma or Ca 15-3 in

breast cancer, CgA has not been proven to be of greater diagnostic and/or prognostic value than the currently used biomarker. It also provides an indication for the presence of a strong component of neuroendocrine differentiation within an adenocarcinoma. That also applies to cases of prostatic adenocarcinoma that develop resistance to androgen deprivation therapy during the progression of the disease, as a result of the gradual shift of the tumor cells towards a neuroendocrine phenotype. The early recognition of that phenomenon may lead to an earlier change in the treatment strategy, which, in turn, may prove to provide clinical benefit. Moreover, it should be used with caution and only in comparison with other methods of determining the course of the disease, such as radiologic and histological evaluation, simply because there are not enough data to support its use as a single, stand-alone marker.

CONCLUSION

Due to the fact that NET symptoms could be vague, or even the disease course may be asymptomatic, diagnosis could be delayed for many years. There is an unmet need for the development of more sensitive, specific and reliable biomarkers for early diagnosis, prognosis and detection of early recurrence to these tumors which comprise an extremely heterogeneous group. Multianalyte assays focusing on novel analytes, such as microRNA, gene transcripts, and circulating tumor cells could be an interesting area for further research given the fact that is unlikely any single marker to be effective.

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Basic Study

Towards automated calculation of evidence-based clinical scores

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Abstract

AIM

To determine clinical scores important for automated calculation in the inpatient setting.

METHODS

A modified Delphi methodology was used to create consensus of important clinical scores for inpatient practice. A list of 176 externally validated clinical scores were identified from freely available internet-based services frequently used by clinicians. Scores were categorized based on pertinent specialty and a customized survey was created for each clinician specialty group. Clinicians were asked to rank each score based on importance of automated calculation to their clinical practice in three categories - "not important", "nice to have", or "very important". Surveys were solicited *via* specialty-group listserv over a 3-mo interval. Respondents must have been practicing physicians with more than 20% clinical time spent in the inpatient setting. Within each specialty, consensus was established for any clinical score with greater than 70% of responses in a single category and a minimum of 10 responses. Logistic regression was performed to determine predictors of automation importance.

RESULTS

Seventy-nine divided by one hundred and forty-four (54.9%) surveys were completed and 72/144 (50%) surveys were completed by eligible respondents. Only the critical care and internal medicine specialties surpassed the 10-responder threshold (14 respondents each). For internists, 2/110 (1.8%) of scores were "very important" and 73/110 (66.4%) were "nice to have". For intensivists, no scores were "very important" and 26/76 (34.2%) were "nice to have". Only the number of medical history (OR = 2.34; 95%CI: 1.26-4.67; $P < 0.05$) and vital sign (OR = 1.88; 95%CI: 1.03-3.68; $P < 0.05$) variables for clinical scores used by internists was predictive of desire for automation.

CONCLUSION

Few clinical scores were deemed "very important" for automated calculation. Future efforts towards score calculator automation should focus on technically feasible "nice to have" scores.

Key words: Automation; Clinical prediction rule; Decision support techniques; Clinical decision support

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Core tip: We report the results of a modified Delphi survey assessing the importance of automated clinical score calculation to practicing internists and intensivists. Although few scores were identified as "very important" for automation, clinicians indicated automated calculation was desired for many commonly used scores. Further studies of the technical feasibility of automating calculation of these scores can help meet these clinicians' needs.

Aakre CA, Dziadzko MA, Herasevich V. Towards automated calculation of evidence-based clinical scores. *World J Methodol* 2017; 7(1): 16-24 Available from: URL: <http://www.wjgnet.com/2222-0682/full/v7/i1/16.htm> DOI: <http://dx.doi.org/10.5662/wjm.v7.i1.16>

INTRODUCTION

Clinical scoring models are ubiquitous in medical literature, but relatively few are routinely used in clinical practice^[1]. In general, models have been created to predict clinical outcomes, to perform risk stratification, to aid in clinical decision making, to assess disease severity, and to assist diagnosis. Clinicians have rejected clinical scoring models for many reasons - they lack external validation, they do not provide clinically useful predictions, they require time-intensive data collection, they involve complex mathematical computations, they use arbitrary categorical cutoffs for clinical predictors, they employ imprecise predictor definitions, they require data elements not routinely collected, or they have poor

accuracy in real practice^[1]. Even among scores accepted by clinicians in clinical practice guidelines^[2-4], these same weaknesses can be barriers to consistent, widespread use.

Score complexity is a frequent barrier to manual calculation, especially given the time constraints of clinical practice. The original APACHE score consisted of 34 physiologic variables; data collection and calculation was time-consuming. Subsequent APACHE scoring models have been simplified to include significantly fewer variables, reducing the risk that needed information was not present^[5-7]. Other popular scores, such as CHADS₂ and HAS-BLED^[8,9], have crafted clever mnemonics and point-based scoring systems for easy use at the point-of-care. Despite these simplifications to support manual calculation, many popular and useful clinical scores have been translated to mobile and internet-based calculators for use at the bedside^[10-12]. Bringing mobile clinical decision support tools to the point-of-care has demonstrated improvements in clinical decision-making^[13], however these tools remain isolated from the clinical data present in the Electronic Health Record (EHR).

In 2009, Congress passed the HITECH act, which aimed to stimulate EHR adoption by hospitals and medical practices. Consequently, as of 2014, 96.9% of hospitals have a certified EHR, and 75.5% have basic EHR capabilities^[14]. Concurrent with EHR adoption, there has been a renewal of the emphasis on improving quality and safety and practicing evidence-based medicine^[15]. Integration of useful evidence-based clinical score models into the EHR with automated calculation based on real-time data is a logical step towards continuing to improve patient care.

The goal of this study is to identify the clinical scores recognized by clinicians as important to the scope of their clinical practice. This information will be invaluable for prioritizing further research into methods of score automation and delivery to the right provider for the right patient in the appropriate clinical context.

MATERIALS AND METHODS

This study was reviewed and approved by the Institutional Review Board at Mayo Clinic in Rochester, MN. This study utilized a modified Delphi methodology to seek a consensus of clinical score calculators important in clinical practice for each represented hospital-based specialty. The Delphi methodology is an iterative process used in studies for the purpose of arriving at a consensus opinion among content experts^[16]. This approach is often utilized when there is incomplete knowledge about a problem or phenomenon and expert judgment is needed for guidance, such as clinical guideline creation^[17]. In general, the Delphi methodology consists of a series of rounds where participating content experts are asked to respond to results from the previous round^[16]. The first round, which serves as a brainstorming session to generate a list of topics for future rounds, can be replaced

Table 1 Description of modified Delphi methodology

Delphi round 1	Systematic collection of online clinical score calculators	Identified 176 externally validated online clinical score calculators
Delphi round 2	Survey development Survey distribution	Branching survey logic mapped score calculators to applicable specialties Academic and community based clinicians

Table 2 Survey respondent characteristics

	Completion rate	n of Scores
Anesthesia	2/5 (40%)	49
Cardiology	1/1 (100%)	37
Critical care	14/23 (61%)	75
Dermatology	0/0	1
Emergency medicine	4/6 (67%)	62
Family medicine	2/5 (40%)	107
Gastroenterology	3/3 (100%)	17
Hematology	1/1 (100%)	5
Infectious disease	2/2 (100%)	2
Internal medicine	14/25 (56%)	109
Nephrology	1/1 (100%)	6
Neurology	0/1 (0%)	23
OBGYN	1/1 (100%)	1
Oncology	1/2 (50%)	5
Orthopedics	0/0	3
Pediatric	7/13 (54%)	25
Pulmonology	4/6 (67%)	17
Surgery	2/3 (67%)	66

by a systematic review in many situations^[16]. The Delphi process used by this study is shown in Table 1.

The list of clinical calculators for the first Delphi round was generated by a prior study performed by our group^[18]. In brief, 176 externally validated clinical scores were identified in calculator form as internet-based services. While this list of clinical calculators is not all-inclusive, it represents all calculators found on popular medical reference web portals (such as Medscape^[11] and UpToDate^[19]) and websites aggregating commonly used clinical calculators^[10-12]. Each calculator was mapped to clinician pertinent specialties for the purpose of generating a customized survey in the next Delphi round. A survey was created in REDCap^[20] utilizing branching logic to ensure that each responding clinician would only be presented a subset of clinical scores pertinent to their specialty. Score-specialty assignment was verified by non-study associated clinicians at our institution in each represented specialty.

In the second Delphi round, the survey was distributed to clinicians in academic and community settings throughout the United States *via* specialty group LISTSERV's. Only practicing clinicians with greater than 20% of their clinical time spent in the inpatient setting were eligible to serve as content experts for this Delphi round. Respondents were asked to assess the importance of automatic calculation of each clinical score to their clinical practice. Each survey item could be ranked on a three-point Likert scale - "not needed", "nice to have", or "very important". Consensus for each score was defined by greater than 70% of clinicians

in each specialty rating the score in any category. A target of at least 10 experts from each represented specialty is recommended to attain consensus based on established Delphi methods^[16]; repeated solicitations were sent to underrepresented specialty groups for 3 mo to maximize participation. Descriptive statistics were obtained for each score, grouped by specialty. Variables for each clinical score were categorized by type of clinical information. Logistic regression was performed to characterize clinical score features predictive of automation importance. Statistical analysis was performed with R version 3.3.1^[21].

RESULTS

One hundred forty-four surveys were initiated by respondents. Seventy-nine in one hundred and forty-four (54.9%) were completed and 72/144 (50.0%) were completed by eligible respondents based on based on level of experience and percent of practice spent in the inpatient setting. Only two specialties, internal medicine and critical care medicine, surpassed the 10-respondent threshold with 14 complete responses each (Table 2). Among internists, only 2/110 (1.8%) were deemed very important for automation, while 73/110 (66.4%) were "nice to have". Among intensivists, no scores were deemed very important for automation, however 26/76 (34.2%) were "nice to have" if automation was possible. A summary of score ratings for both specialties can be found in Table 3. Suggestions of missing scores included Centor criteria, Ottawa knee/ankle/foot rules, estimated free water deficit, opioid risk assessment tool, Bishop score, and several screening questionnaires. Too few scores were ranked as "very important" for automation by either specialty to perform regression, however logistic regression was performed on a composite outcome of scores deemed "nice to have" + "very important" (Table 4).

DISCUSSION

This study assesses clinicians' perspectives on the importance of automating specific clinical scores within the EHR for their clinical practice. We chose a modified Delphi methodology because of our previous study's thoroughness in identifying clinical score calculators across multiple specialty domains and to reduce respondent survey burden. The primary advantage of using a modified Delphi methodology in this study is the ability to capture the valuation of multiple scores by clinicians across varying specialties. The primary disadvantage to this methodology is the recruitment of appropriate content

Table 3 Summary of importance of automation of specified clinical scores ranked by critical care and internal medicine physicians

Score name	Year of creation	<i>n</i> of variables	Very important	Very important or nice to have
Critical care				
APACHE II	1985	15	9/14 (64.3%)	12/14 (85.7%)
SNAP II	2001	9	7/11 (63.6%)	9/11 (81.8%)
NRDS scoring system	1998	5	7/12 (58.3%)	10/12 (83.3%)
Post-anesthetic recovery score	1970	5	7/12 (58.3%)	9/12 (75%)
Rotterdam score	1997	4	7/12 (58.3%)	8/12 (66.7%)
SNAP	1993	27	7/12 (58.3%)	9/12 (75%)
SNAP-PE	1993	30	7/12 (58.3%)	9/12 (75%)
SNAP-PE II	2001	12	7/12 (58.3%)	9/12 (75%)
Wells criteria for DVT	2006	9	7/12 (58.3%)	9/12 (75%)
Wells criteria for PE	1998	7	7/12 (58.3%)	10/12 (83.3%)
PAWS	2008	7	6/11 (54.5%)	8/11 (72.7%)
CRIB	1993	5	6/12 (50%)	8/12 (66.7%)
CRIB II	2003	5	6/12 (50%)	8/12 (66.7%)
MSSS	2002	7	6/12 (50%)	8/12 (66.7%)
PELOD score	1999	13	3/6 (50%)	4/6 (66.7%)
SAPS II	1993	16	5/10 (50%)	7/10 (70%)
TIMI risk index	2006	3	5/11 (45.5%)	8/11 (72.7%)
TRISS	1987	9	4/9 (44.4%)	6/9 (66.7%)
Children's coma score	1984	3	3/7 (42.9%)	4/7 (57.1%)
PRISM score	1988	16	3/7 (42.9%)	5/7 (71.4%)
CURB-65	2003	5	5/12 (41.7%)	8/12 (66.7%)
SCORETEN scale	2000	6	5/12 (41.7%)	9/12 (75%)
MEWS score	2006	6	4/10 (40%)	6/10 (60%)
Rockall score	2008	11	3/8 (37.5%)	5/8 (62.5%)
TRIOS score	2001	4	3/8 (37.5%)	5/8 (62.5%)
Geneva score for PE	2006	9	4/11 (36.4%)	7/11 (63.6%)
Injury Severity Score	1974	6	4/11 (36.4%)	8/11 (72.7%)
Lung Injury score	1988	5	4/11 (36.4%)	8/11 (72.7%)
MPMII - admission	1993	14	4/11 (36.4%)	6/11 (54.5%)
MPMII - 24-48-72	1993	14	4/11 (36.4%)	6/11 (54.5%)
LODS score	1996	12	3/9 (33.3%)	7/9 (77.8%)
MEDS score	2003	10	3/9 (33.3%)	6/9 (66.7%)
MESS score	1990	5	4/12 (33.3%)	7/12 (58.3%)
Parsonnet Score	1989	14	4/12 (33.3%)	7/12 (58.3%)
Pediatric coma scale	1988	3	2/6 (33.3%)	3/6 (50%)
RAPS	1987	5	3/9 (33.3%)	7/9 (77.8%)
Surgical Apgar score	2007	3	4/12 (33.3%)	8/12 (66.7%)
ASCOT score	1990	8	4/13 (30.8%)	6/13 (46.2%)
MELD score	2001	4	4/13 (30.8%)	12/13 (92.3%)
PIM2	2003	8	2/7 (28.6%)	5/7 (71.4%)
SWIFT score	2008	6	2/7 (28.6%)	4/7 (57.1%)
Clinical Pulmonary Infection Score	1991	8	3/11 (27.3%)	9/11 (81.8%)
MPM-24 h	1988	15	3/11 (27.3%)	6/11 (54.5%)
Child-Pugh Score	1973	5	3/12 (25%)	11/12 (91.7%)
Decaf score	2012	5	2/8 (25%)	4/8 (50%)
ONTARIO score	1995	6	2/8 (25%)	4/8 (50%)
AKICS score	2007	8	3/13 (23.1%)	7/13 (53.8%)
AVPU scale	2004	4	2/9 (22.2%)	6/9 (66.7%)
PERC rule for PE	2001	7	2/9 (22.2%)	6/9 (66.7%)
RIETE score	1988	6	2/9 (22.2%)	6/9 (66.7%)
BISAP score for pancreatitis mortality	2008	5	2/10 (20%)	4/10 (40%)
Bleeding risk score	2007	4	2/10 (20%)	6/10 (60%)
Clinical asthma evaluation score	1972	5	2/10 (20%)	6/10 (60%)
PIRO score	2009	8	2/10 (20%)	7/10 (70%)
ABC score for massive transfusion	2009	4	2/11 (18.2%)	6/11 (54.5%)
ACLS score	1981	4	2/11 (18.2%)	7/11 (63.6%)
MOD score	1995	7	2/11 (18.2%)	8/11 (72.7%)
MPM - admission	1988	10	2/11 (18.2%)	6/11 (54.5%)
sPESI	2010	8	2/11 (18.2%)	7/11 (63.6%)
ABIC score	2008	4	2/12 (16.7%)	5/12 (41.7%)
CRUSADE score	2009	8	2/12 (16.7%)	6/12 (50%)
Pediatric trauma score	1988	6	1/6 (16.7%)	2/6 (33.3%)
LRINEC Score for Necrotizing STI	2004	5	1/8 (12.5%)	4/8 (50%)
Panc 3 score	2007	3	1/8 (12.5%)	3/8 (37.5%)
Pancreatitis outcome score	2007	7	1/8 (12.5%)	3/8 (37.5%)
TASH score	2006	7	1/8 (12.5%)	4/8 (50%)

POSSUM score	1991	18	1/9 (11.1%)	3/9 (33.3%)
Revised Trauma score	1981	3	1/9 (11.1%)	5/9 (55.6%)
24 h ICU trauma score	1992	4	1/10 (10%)	7/10 (70%)
HIT Expert Probability Score	2010	11	1/11 (9.1%)	6/11 (54.5%)
Bronchiectasis severity index	2014	10	1/12 (8.3%)	4/12 (33.3%)
Oxygenation index	2005	3	1/13 (7.7%)	7/13 (53.8%)
CT severity index	1990	1	0/12 (0%)	6/12 (50%)
Glasgow coma scale	1974	3	0/13 (0%)	10/13 (76.9%)
SOFA	2001	6	0/13 (0%)	8/13 (61.5%)
Internal medicine				
Wells criteria for DVT	2006	9	10/14 (71.4%)	13/14 (92.9%)
Wells criteria for PE	1998	7	10/14 (71.4%)	13/14 (92.9%)
CHA2DS2-VASc	2010	7	9/14 (64.3%)	13/14 (92.9%)
TIMI risk index	2006	3	9/14 (64.3%)	13/14 (92.9%)
TIMI risk score for UA/NSTEMI	2000	7	9/14 (64.3%)	13/14 (92.9%)
TIMI risk score for STEMI	2000	9	9/14 (64.3%)	13/14 (92.9%)
CURB-65	2003	5	8/14 (57.1%)	13/14 (92.9%)
STESS score	2008	4	8/14 (57.1%)	13/14 (92.9%)
Duke criteria for IE	1994	8	6/13 (46.2%)	12/13 (92.3%)
PESI	2006	11	7/12 (58.3%)	11/12 (91.7%)
Revised cardiac risk index for pre-operative risk	1999	6	7/12 (58.3%)	11/12 (91.7%)
SOFA	2001	6	6/12 (50%)	11/12 (91.7%)
ABCD2 score	2006	5	5/12 (41.7%)	11/12 (91.7%)
Charlson Comorbidity index	1987	1	2/12 (16.7%)	11/12 (91.7%)
PERC rule for PE	2001	7	5/11 (45.5%)	10/11 (90.9%)
sPESI	2010	8	4/11 (36.4%)	10/11 (90.9%)
MOD score	1995	7	3/11 (27.3%)	10/11 (90.9%)
MPM - 24 h	1988	15	4/10 (40%)	9/10 (90%)
MPM - admission	1988	10	3/10 (30%)	9/10 (90%)
MEDS score	2003	10	2/10 (20%)	9/10 (90%)
PIRO score	2009	8	1/10 (10%)	9/10 (90%)
SAPS II	1993	16	4/9 (44.4%)	8/9 (88.9%)
SWIFT score	2008	6	2/8 (25%)	7/8 (87.5%)
Panc 3 score	2007	3	1/8 (12.5%)	7/8 (87.5%)
APACHE II	1985	15	9/14 (64.3%)	12/14 (85.7%)
Parsonnett Score	1989	14	8/14 (57.1%)	12/14 (85.7%)
HIT Expert Probability Score	2010	11	6/14 (42.9%)	12/14 (85.7%)
Ranson's criteria	1974	11	6/14 (42.9%)	12/14 (85.7%)
TRIOS score	2001	4	3/7 (42.9%)	6/7 (85.7%)
4Ts Score	2006	5	5/14 (35.7%)	12/14 (85.7%)
Framingham coronary heart disease risk score	1998	7	5/14 (35.7%)	12/14 (85.7%)
30 d PCI readmission risk	2013	10	2/7 (28.6%)	6/7 (85.7%)
Glasgow coma scale	1974	3	9/13 (69.2%)	11/13 (84.6%)
Modified NIH Stroke Scale	2001	9	7/13 (53.9%)	11/13 (84.6%)
King's College Criteria for Acetaminophen Toxicity	1989	6	4/12 (33.3%)	10/12 (83.3%)
Glasgow-Blatchford Bleeding score	2000	9	3/12 (25%)	10/12 (83.3%)
ATRIA bleeding risk score	2011	6	2/12 (16.7%)	10/12 (83.3%)
Glasgow Alcoholic hepatitis score	2005	4	5/11 (45.5%)	9/11 (81.8%)
MEWS score	2006	6	4/11 (36.4%)	9/11 (81.8%)
Hemorrhages score	2006	11	2/11 (18.2%)	9/11 (81.8%)
Decaf score	2012	5	4/10 (40%)	8/10 (80%)
MPMII - admission	1993	14	4/10 (40%)	8/10 (80%)
MPMII - 24-48-72	1993	14	4/10 (40%)	8/10 (80%)
Malnutrition universal screening tool (MUST)	2004	3	2/10 (20%)	8/10 (80%)
ASTRAL score	2012	6	1/10 (10%)	8/10 (80%)
GRACE ACS	2006	12	1/10 (10%)	8/10 (80%)
CHADS2	2001	5	7/14 (50%)	11/14 (78.6%)
Multidimensional frailty score	2014	9	7/14 (50%)	11/14 (78.6%)
Geneva score for PE	2006	9	3/9 (33.3%)	7/9 (77.8%)
Pittsburg knee rules	1994	3	3/9 (33.3%)	7/9 (77.8%)
Mayo scoring system for assessment of ulcerative colitis activity	2005	4	1/9 (11.1%)	7/9 (77.8%)
4-yr mortality prognostic index	2006	12	1/9 (11.1%)	7/9 (77.8%)
Rockall score	2008	11	1/9 (11.1%)	7/9 (77.8%)
SHARF scoring system	2004	9	1/9 (11.1%)	7/9 (77.8%)
HAS-BLED	2010	12	5/13 (38.5%)	10/13 (76.9%)
ATRIA stroke risk score	2013	7	3/12 (25%)	9/12 (75%)
Euroscore	1999	17	1/8 (12.5%)	6/8 (75%)
Renal risk score	2011	6	1/8 (12.5%)	6/8 (75%)
ROSE risk score	1996	7	1/8 (12.5%)	6/8 (75%)
LRINEC Score for Necrotizing STI	2004	5	3/11 (27.3%)	8/11 (72.7%)

Bleeding risk score	2007	4	2/11 (18.2%)	8/11 (72.7%)
CT severity index	1990	1	1/11 (9.1%)	8/11 (72.7%)
SCORETEN scale	2000	6	7/14 (50%)	10/14 (71.4%)
REMS	2004	7	2/7 (28.6%)	5/7 (71.4%)
Mayo CABG risk of inpatient death after MI	2007	7	1/7 (14.3%)	5/7 (71.4%)
Mayo PCI risk of inpatient MACE	2007	7	1/7 (14.3%)	5/7 (71.4%)
QMMI score	2001	11	1/7 (14.3%)	5/7 (71.4%)
MELD score	2001	4	0/14 (0%)	10/14 (71.4%)
Nexus criteria for C-spine imaging	1970	5	4/10 (40%)	7/10 (70%)
Birmingham nutritional risk score	1995	7	2/10 (20%)	7/10 (70%)
Canadian CT head rule	2001	9	2/10 (20%)	7/10 (70%)
ACLS score	1981	4	1/10 (10%)	7/10 (70%)
San Francisco syncope rule	2004	5	1/10 (10%)	7/10 (70%)
Mannheim peritonitis index	1993	7	6/13 (46.2%)	9/13 (69.2%)
HADO score	2006	4	3/9 (33.3%)	6/9 (66.7%)
CARE score	2001	3	1/9 (11.1%)	6/9 (66.7%)
ICH score	2001	5	1/9 (11.1%)	6/9 (66.7%)
Adult appendicitis score	2014	8	6/14 (42.9%)	9/14 (64.3%)
IMPACT score	2008	11	6/14 (42.9%)	9/14 (64.3%)
CRUSADE score	2009	8	4/14 (28.6%)	9/14 (64.3%)
PORT/PSI score	1997	20	2/14 (14.3%)	9/14 (64.3%)
CIWA-Ar	1989	10	1/14 (7.1%)	9/14 (64.3%)
LODS score	1996	12	3/8 (37.5%)	5/8 (62.5%)
OESIL risk score	2003	4	2/8 (25%)	5/8 (62.5%)
QRISK2	2010	14	2/8 (25%)	5/8 (62.5%)
Qstroke score	2013	15	2/8 (25%)	5/8 (62.5%)
RIETE score	1988	6	2/8 (25%)	5/8 (62.5%)
EGSYS score	2008	6	1/8 (12.5%)	5/8 (62.5%)
EHMRG	2012	10	1/8 (12.5%)	5/8 (62.5%)
FOUR score	2005	4	1/8 (12.5%)	5/8 (62.5%)
Pancreatitis outcome score	2007	7	1/8 (12.5%)	5/8 (62.5%)
Prostate cancer prevention trial risk calculator	1993	6	6/13 (46.2%)	8/13 (61.5%)
Alvarado score for acute appendicitis	1986	8	5/13 (38.5%)	8/13 (61.5%)
DRAGON score	2012	6	1/10 (10%)	6/10 (60%)
Bronchiectasis severity index	2014	10	3/14 (21.4%)	8/14 (57.1%)
New Orleans head CT rule	2000	8	1/7 (14.3%)	4/7 (57.1%)
POSSUM score	1991	18	1/7 (14.3%)	4/7 (57.1%)
Child-Pugh Score	1973	5	0/14 (0%)	8/14 (57.1%)
Lung Injury score	1988	5	4/9 (44.4%)	5/9 (55.6%)
AVPU scale	2004	4	2/9 (22.2%)	5/9 (55.6%)
Gupta perioperative cardiac risk	2011	5	2/9 (22.2%)	5/9 (55.6%)
HEART score	2008	5	1/9 (11.1%)	5/9 (55.6%)
IgA nephropathy score	2006	8	5/14 (35.7%)	7/14 (50%)
ABIC score	2008	4	4/14 (28.6%)	7/14 (50%)
CAMBS score	1993	4	4/14 (28.6%)	7/14 (50%)
GAP risk assessment score	2012	4	2/8 (25%)	4/8 (50%)
BISAP score for pancreatitis mortality	2008	5	2/10 (20%)	5/10 (50%)
ONTARIO score	1995	6	1/8 (12.5%)	4/8 (50%)
JAMA kidney failure risk equation	2011	7	4/13 (30.8%)	5/13 (38.5%)

experts for each Delphi round^[16]. Because this study focused on the automated calculation of scores used in inpatient clinical practice, we limited analysis to board-certified clinicians practicing more than 20% of their time in the inpatient setting. This requirement allowed use to gather diverse viewpoints of practicing clinicians in various practice settings.

Clinical scores can play important roles in the clinical decision-making algorithms used daily by clinicians. Mobile and internet-based clinical calculators have made these daily clinical score calculations easier; however the use of these standalone technologies does not reduce the time and effort required for manual data retrieval and entry. Automated retrieval of variables required for score calculation within the EHR eliminates the need for these potentially workflow disrupting standalone smartphone or

web applications^[22]. Additionally, automated calculation of clinical scores provides a mechanism to improve care standardization, to facilitate adherence to evidence-based practice and clinical guidelines, and to save time^[1]. However, just as clinicians have rejected many clinical scores for routine usage, our study found that clinicians did not appraise most clinical scores as “very important” for automation.

The clinical score variables examined in this study spanned several broad categories - demographic information, laboratory values, medical history elements, clinical examination findings, clinical judgments, and even other clinical scores. Some categories, such as laboratory values or medical history elements, may require more time-intensive data retrieval compared to others. We predicted that commonly used scores with cognitively

Table 4 Predictors of desirability of score automation based on number of each variable type in each score

Automation: Very important/nice to have	OR (95%CI)
Critical care	
<i>n</i> of variables	0.68 (0.23, 1.59)
Clinical history	1.36 (0.36, 4.93)
Vital sign	1.40 (0.53, 4.6)
Medication	4.89 (0.10, 237.52)
Clinical judgment	2.33 (0.76, 9.80)
Examination	0.99 (0.36, 3.14)
Laboratory value	1.48 (0.61, 4.41)
Charted variable (non-vital)	2.26 (0.70, 8.93)
Demographic value	0.20 (0.03, 1.00)
Another score	2.07 (0.39, 12.13)
Internal medicine	
<i>n</i> of variables	0.64 (0.39, 1.04)
Clinical history	2.34 ^a (1.26, 4.67)
Vital sign	1.88 ^a (1.03, 3.68)
Medication	2.89 (0.37, 63.17)
Clinical judgment	1.41 (0.75, 2.74)
Examination	1.56 (0.88, 2.87)
Laboratory value	1.51 (0.90, 2.62)
Charted variable (non-vital)	2.54 (0.85, 8.70)
Demographic value	0.90 (0.41, 1.97)
Another score	0.89 (0.30, 2.17)

^a*P* < 0.05.

demanding information extraction would be more desirable for automation. However, our regression model did not explicitly include variables representing time-required for data collection or data entry for any score - the key efficiencies gained through automated calculation. Instead, we used the number of variables in the score and variable categorization as surrogates to account for these cognitively demanding tasks. No association between the number of clinical variables and desirability of automation was found for the internal medicine or critical care specialties. Only two scores met the threshold for being "very important" for automation by internists - Wells criteria for DVT^[23] (10/13, 71.4%) and PE^[24] (10/13, 71.4%). Although many more scores were deemed "nice to have" by both specialties, regression analysis only identified the number of medical history variables (OR = 2.34; 95%CI: 1.26-4.67; *P* < 0.05) and vital sign variables (OR = 1.88; 95%CI: 1.03-3.68; *P* < 0.05) as predictive of desirability of automation among internists. The time and cognitive workload of performing manual chart review for unknown aspects of the medical history may explain this finding; several tools have been created to meet this clinical need^[25,26].

The time-benefit gained from reduced workflow disruption may be more apparent in scores pertaining to common clinical scenarios, such as sepsis. During the survey period, the SOFA score was integrated into the operational definition of sepsis^[17], likely affecting the valuation of automated calculation by some specialties. The prospective benefit of automated calculation of this and similar scores is readily apparent; one study comparing automated and manual calculation of the SOFA score^[27] found an average time-savings of about 5 min per

calculation attained by automation^[28]. Extrapolated to a unit of 12 patients, up to one hour of work could be saved daily through automated calculation of this single score. More complex scores may have even greater time-savings.

This study has several limitations. First, the survey items may not represent all pertinent clinical scores in all specialties surveyed. We did consult with local experts in each specialty to review the completeness of the list of clinical scores. Additionally, respondents were solicited for additional scores to be considered. Many of the suggestions represented either diagnostic criteria (Centor criteria or Ottawa foot/ankle/knee rules) or diagnostic questionnaires (PHQ-9, CAGE, AUDIT) - all are useful clinical tools but not amenable to automated score calculation.

Second, the responding experts may not represent the viewpoints of all clinicians in each field. We sought a heterogeneous group of clinicians within each specialty, representing both academic and community hospital settings nationwide. However, only 6 internists and 6 intensivists that completed our survey volunteered their hospital's name; all were academic health centers. This potential response bias would favor clinical scores used primarily in academic settings, a concern that has been raised for certain scores^[29]. Additionally, survey response rate was low despite multiple solicitations targeting lesser represented specialties, a likely reflection of physician survey fatigue.

Third, consensus was not reached for most clinical scores for either specialty. Since both specialties had a large number of pertinent clinical scores, it would be expected that consensus could not be reached for many scores. When exploring the programmability of specific clinical scores, researchers may be more inclined to investigate methods for automated calculation of "nice to have" scores that are highly programmable to meet the needs of these clinicians. Further investigation is needed to assess the overall programmability of each clinical score calculator within modern electronic medical record systems utilizing commonly available clinical data and information retrieval techniques.

In conclusion, Internal medicine and critical care physicians assessed evidence-based clinical scores on the importance of automated calculation to their clinical practice. Very few clinical scores were deemed "very important" to automate, while many were considered "nice to have". In order to prioritize automating calculation of some of these "nice to have" clinical scores, further research is needed to evaluate the feasibility of programming each score in the electronic medical record.

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COMMENTS

Background

Numerous clinical scores have been created, but it is not known which scores may be important for automated calculation within the electronic medical record.

Research frontiers

Automated calculation of important scores can reduce physician's cognitive workload and facilitate practice guideline adherence.

Innovations and breakthroughs

This study is a comprehensive assessment of importance of automating calculation of clinical scores in the inpatient setting.

Applications

In this study, clinicians identified specific clinical scores as desirable for automated calculation. This information can guide future research on techniques to automate these scores to meet clinician's needs.

Peer-review

The authors investigated scoring systems of evidence for clinical application. The aim was clear and results were useful.

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Observational Study

Patch testing and cross sensitivity study of adverse cutaneous drug reactions due to anticonvulsants: A preliminary report

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Abstract

AIM

To evaluate the utility of patch test and cross-sensitivity patterns in patients with adverse cutaneous drug reactions (ACDR) from common anticonvulsants.

METHODS

Twenty-four (M:F = 13:11) patients aged 18-75 years with ACDR from anticonvulsants were patch tested 3-27 mo after complete recovery using carbamazepine, phenytoin, phenobarbitone, lamotrigine, and sodium valproate in 10%, 20% and 30% conc. in pet. after informed consent. Positive reactions persisting on D3 and D4 were considered significant.

RESULTS

Clinical patterns were exanthematous drug rash with or without systemic involvement (DRESS) in 18 (75%), Stevens-Johnsons syndrome/toxic epidermal necrolysis (SJS/TEN) overlap and TEN in 2 (8.3%) patients each, SJS and lichenoid drug eruption in 1 (4.2%) patient each, respectively. The implicated drugs were phenytoin in 14 (58.3%), carbamazepine in 9 (37.5%), phenobarbitone in 2 (8.3%), and lamotrigine in 1 (4.7%) patients,

respectively. Twelve (50%) patients elicited positive reactions to implicated drugs; carbamazepine in 6 (50%), phenytoin alone in 4 (33.3%), phenobarbitone alone in 1 (8.3%), and both phenytoin and phenobarbitone in 1 (8.33%) patients, respectively. Cross-reactions occurred in 11 (92%) patients. Six patients with carbamazepine positive patch test reaction showed cross sensitivity with phenobarbitone, sodium valproate and/or lamotrigine. Three (75%) patients among positive phenytoin patch test reactions had cross reactions with phenobarbitone, lamotrigine, and/or valproate.

CONCLUSION

Carbamazepine remains the commonest anticonvulsant causing ACDRs and cross-reactions with other anticonvulsants are possible. Drug patch testing appears useful in DRESS for drug imputability and cross-reactions established clinically.

Key words: Anticonvulsant hypersensitivity syndrome; Carbamazepine; Sodium valproate; Drug rash with eosinophilia with or without systemic involvement; Drug patch test; Lamotrigine; Phenobarbitone; Phenytoin; Stevens-Johnsons syndrome; Toxic epidermal necrolysis

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Core tip: Anticonvulsants account for 20% of all adverse cutaneous drug reactions (ACDRs) while cross-reactions occur frequently among carbamazepine, phenytoin, phenobarbitone necessitating careful prescriptions. The clinical presentation alone is not diagnostic and identification of offending drug needs causality assessment that may be misleading in patients on multiple medications. Drug provocation, skin prick or intradermal tests have ethical issues for possibility of precipitating more severe reactions. Basophil degranulation/lymphocyte activation or drug specific IgE radioallergosorbent tests, histamine release and passive haemagglutination tests have limited use in clinical practice. Drug patch testing appears useful in anticonvulsant ACDRs, drug imputability and cross-reactions established clinically.

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INTRODUCTION

Adverse cutaneous drug reaction (ACDR) is a frequent problem in clinical practice comprising 1%-2% of out-door and 6%-30% of indoor patients in dermatology. ACDRs from anticonvulsants [carbamazepine, phenytoin, phenobarbitone (aromatic group), lamotrigine and so-

dium valproate] account for 20% of all drug rashes^[1]. Lamotrigine itself is associated with high adverse cutaneous reactions in 10% or more cases and its combination with sodium valproate further enhances this risk. They cause transient maculopapular rash that may eventuate to more severe life threatening adverse cutaneous reactions like exanthematous drug hypersensitivity, drug rash with eosinophilia with or without systemic involvement (DRESS), Stevens-Johnsons syndrome/toxic epidermal necrolysis (SJS/TEN) collectively known as anticonvulsant hypersensitivity syndrome^[2]. Cross-reactions especially aromatic anticonvulsants (carbamazepine, phenytoin, phenobarbitone), lamotrigine, and sodium valproate frequently makes selection of an alternative agent difficult^[3]. The focus has shifted in recent years on the utility of drug patch test in cutaneous adverse drug reactions for ease and positive results can be useful to confirm drug imputability established on clinical grounds. Moreover, the risk with patch testing is considerably lower when compared to intracutaneous or oral provocation tests. Although the reliability of patch testing in identification of the culprit drug has been reported^[4], the cross-reactions among anticonvulsants remain under studied. This study intended to evaluate the utility of patch test in patients with ACDRs from anticonvulsants and occurrence of cross-sensitivity patterns among these drugs.

MATERIALS AND METHODS

Twenty four patients diagnosed and treated previously for ACDRs from anticonvulsants were patch tested after informed consent between April 2014 and March 2015 when they were off systemic treatments including corticosteroids for ≥ 4 wk. Pregnant and lactating women, children aged under 18 years, patients with recent acute reaction, suspected viral exanthem or autoimmune disorders, and who were using topical corticosteroids over the back within the last one week were excluded from the study. Clinical details of age, gender, onset, duration and progress of drug rash, the suspected offending anticonvulsant drug, all treatments taken before or after onset of rash, personal and type of ACDRs were recorded.

Since pure form of drugs could not be obtained, antigens for patch testing were prepared as suggested by Friedmann and Arden-Jones^[5] from pulverized prescribable tablets of carbamazepine, phenytoin, phenobarbitone, lamotrigine, and sodium valproate in petrolatum having active drug in 10%, 20%, 30% conc. The patch test was performed by Finn chamber (7 mm) method as described previously using 0.02 mL of test antigen^[4]. The patch tests were applied on dry, non-hairy upper back after cleansing with ethanol. The patients returned for reading of results after 48 h (D2), 72 h (D3) and 96 h (D4) and results were graded as per International Contact Dermatitis Research Group criteria^[6]. Reactions persisting on D3 or D4 were considered significant for final analysis. None of the test concentration elicited irritant/

Table 1 Baseline characteristics of all patients

Baseline characteristics	Number of patients underwent patch testing <i>n</i> = 24 (%)
Gender	
Male	13 (54.2)
Female	11 (45.8)
M:F	1:1.8
Age (yr)	
Range	18-75
Mean \pm SD	45.70 \pm 16.29
18-30	4 (16.7)
31-50	12 (50)
51-70	6 (25)
> 70	2 (8.3)
Time interval (d) between drug intake and ACDRs	
Range	7-45
Mean \pm SD	22.54 \pm 12.19
Implicated drugs	
Phenytoin	14 (58.3)
Carbamazepine	9 (37.5)
Phenobarbitone	2 (8.3)
Lamotrigine	1 (4.7)
Phenytoin ¹ + Carbamazepine	1 (4.7)
Phenytoin ¹ + Phenobarbitone	1 (4.7)
Clinical spectrum of ACDRs	
DRESS	18 (75)
SJS-TEN overlap	2 (8.3)
TEN	2 (8.3)
SJS	1 (4.2)
Lichenoid drug eruption	1 (4.2)
Time interval (mo) between complete recovery from ACDRs and Patch test	
Range	1-24
Mean \pm SD	9.62 \pm 6.62

¹Also included in 14 patients with ACDRs from Phenytoin. ACDRs: Adverse cutaneous drug reactions; DRESS: Drug rash with eosinophilia with or without systemic involvement; SJS: Stevens-Johnsons syndrome; TEN: Toxic epidermal necrolysis.

allergic reaction in ten healthy adult volunteers in prior testing. The relevance of positive patch test results was determined clinically. Any side effects from patch testing (adhesive tape reaction, itching/flare up, angry back phenomenon, or pigment alteration) were noted.

RESULTS

Tables 1 and 2 lists baseline characteristics of study patients, incubation period, common clinical patterns of ACDRs observed, individual implicated anticonvulsants, and time interval between complete recovery from ACDR and drug patch test. The majority, 14 (58.3%) patients were of DRESS and nine were from phenytoin (Figure 1). None of them had received any drug(s) other than anticonvulsant(s) before or after the onset of drug rash.

Only 12 (50%) patients had positive patch test reactions from the primarily implicated drug and/or other anticonvulsants 4-9 mo after complete recovery from ACDR (Table 3). Carbamazepine elicited positive reactions in 6 of 8 patients with carbamazepine hypersensitivity

Table 2 Clinical patterns of adverse cutaneous drug reaction and individual implicated anticonvulsants

Implicated drugs	Clinical patterns (<i>n</i> = 24)				
	DRESS	SJS	SJS-TEN overlap	TEN	Lichenoid drug eruption
Phenytoin	9	1	1	1	-
Carbamazepine	6	-	-	1	1
Phenytoin + Carbamazepine	-	-	1	-	-
Phenytoin + Phenobarbitone	1	-	-	-	-
Lamotrigine	-	-	1	-	-
Phenobarbitone	1	-	-	-	-
Sodium valproate + Lamotrigine	-	-	-	-	-

DRESS: Drug rash with eosinophilia with or without systemic involvement; SJS: Stevens-Johnsons syndrome; TEN: Toxic epidermal necrolysis.

and cross reactions from one or more drugs that included sodium valproate (3 patients), lamotrigine (4 patients), and phenobarbitone (2 patients), respectively (Figure 2). Similarly, phenytoin elicited positive reactions in 4 of 11 patients with phenytoin hypersensitivity. Cross-reactions were also observed in 3 patients from phenobarbitone (2 patients), and sodium valproate and lamotrigine in one patient. Phenobarbitone that had caused DRESS in one patient also elicited positive reaction in him along with cross sensitivity to carbamazepine, phenytoin, sodium valproate and lamotrigine. One patient with DRESS from combination of phenytoin and phenobarbitone showed positivity to both the drugs and cross sensitivity with lamotrigine. Lamotrigine in 7, carbamazepine, phenytoin in 6 patients each elicited more number of positive reactions with 30% concentration than their 20% and 10% concentrations. Patch test positivity from phenobarbitone (in 6 patients) or sodium valproate (in 4 patients) was more with 10% concentration than from their higher concentrations. Sodium valproate elicited positive reaction with all concentrations but more so with 10% and 30% (4 patients each) as compared to 20% eliciting positive reactions in two patients only (Table 4).

Overall, 24 irritant reactions were observed in nine patients (Table 3). These were from sodium valproate in 6 patients (4 reactions each from 10%, 20%, and 30%), carbamazepine (2 reactions from 10%, and one reaction each from 20% and 30%) and phenytoin in 3 patients each (2 reactions from 10% and one reaction from 20%). Phenobarbitone (one from 10%, and two reactions from 30%), and lamotrigine (two reactions from 10%) elicited irritant reactions in 2 patients each. The irritant reactions from sodium valproate in two patients were from all three concentrations lasting for > 72 h. No patient had patch test related side effects.

DISCUSSION

The patch testing is a preferred investigation in adverse ACDRs as well as it helps in studying cross-reactions and understanding the pathomechanisms of drug eru-

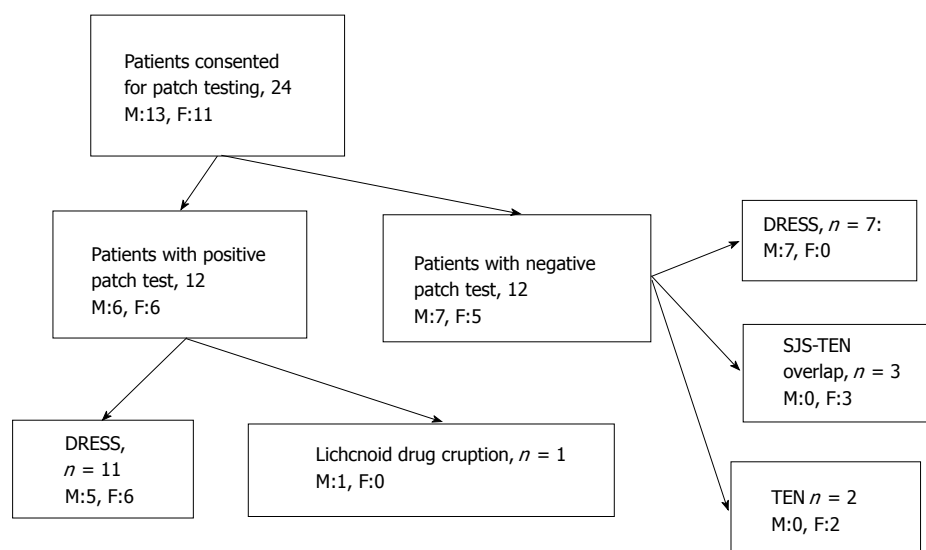


Figure 1 Attributes of 24 study patients for drug patch testing at a glance.

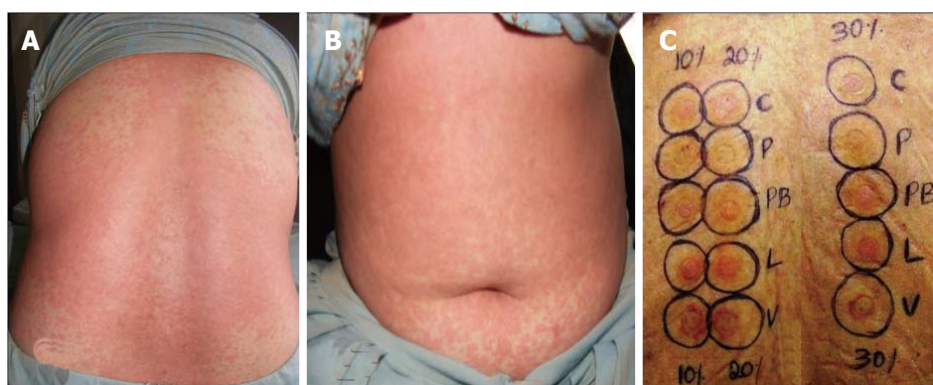


Figure 2 A patient of drug rash with eosinophilia and systemic symptoms from carbamazepine having prominently macular erythema and pruritic maculopapular rash over trunk (A) back, (B) front. She also had facial edema, conjunctival congestion and chemosis (not in picture); (C) Drug patch tests reactions (1+) reaction from carbamazepine (10%) and cross reactions (2+) from lamotrigine (10%, 20%, 30%), and (1+) from phenobarbitone (10%, 20%, 30%). Sodium valproate (10%, 20% and 30%) has elicited irritant reactions.

ptions that is essentially same as that in patch testing for allergic contact dermatitis^[4]. Briefly, it is type-4 (delayed type) hypersensitivity involving CD4 or CD8 T-lymphocytes producing different patterns of cytokines and/or cytotoxic factors. The antigen (drug molecule or the metabolite, the hapten, and protein complex) is presented to T-helper cells after processing by antigen presenting cells. The T-helper cells after getting activated proliferate and produce clones of specific immunogenic memory/effector T-cells having ability to activate immune effector mechanism (immunological memory) as well as help antibody (IgA, IgG, IgE) production from B cells during this sensitization phase lasting for 7-10 d. This is followed by elicitation phase when the offending drug will elicit similar clinical reaction on re-exposure and positive patch test reactions in individuals sensitized previously.

Carbamazepine, phenytoin, phenobarbitone and lamotrigine, alone or in combination, may induce ACDs such as DRESS, SJS, SJS-TEN overlap, and TEN. Arene oxide metabolites from a shared metabolic pathway

of carbamazepine, phenytoin and phenobarbitone, the commonest offending aromatic drugs, have been implicated in the pathogenesis of hypersensitivity reactions and cross reactivity among these anticonvulsants. Sodium valproate inhibits metabolism of lamotrigine and increases the risk of severe ACDs. Frequency of positive drug patch tests varies between 7% and 87% in ACDs from groups of drugs including anticonvulsants across studies^[1,7-11]. The patch test positivity of 50% in the present study is comparable. The significance of drug patch test in SJS/TEN and exfoliative dermatitis due to anticonvulsants remains poorly elucidated since these patients usually elicit no or weak positive reactions. Contrarily, highest patch test positivity occurs in maculopapular/exanthematous drug rash such as DRESS^[1,12] as was also observed in our 11 (92%) of 12 patients with DRESS. It is possibly due to the pathomechanism (Th2 cytokine response) involved in DRESS that differs from that in SJS/TEN (cytotoxic T-cell response). Carbamazepine has been the commonest

Table 3 Positive drug patch test results

Case No	Age (yr) and Sex	Clinical diagnosis	Implicated drug	Interval between drug rash and patch test (mo)	Patch test results (Grades)	Cross reactions (Grades)	Irritant reaction at D2
1	65 F	DRESS	Carbamazepine	7	Carbamazepine (2+) with 10%, 20%, 30%	Sodium valproate (1+) with 20%, 30%	-
2	60 F	DRESS	Carbamazepine	6	Carbamazepine (3+) with 20%, 30%	Lamotrigine (3+) with 30%	Carbamazepine 10%, Lamotrigine 10%, Sodium valproate 10%, 30% Phenobarbitone 30%
3	55 F	DRESS	Carbamazepine	5	Carbamazepine (1+) with 30%	Sodium valproate (2+) with 10%, 30% Lamotrigine (1+) with 10%, 20%, 30%	Carbamazepine 10% and 20%, Phenobarbitone 10%, 30% and Sodium valproate 20%
4	52 M	Lichenoid drug eruptions	Carbamazepine	6	Carbamazepine (2+) with 10%, 20%, 30%	Phenobarbitone (2+) with 10%, 20%, 30%	Phenytoin 20%, Sodium valproate 20% and Lamotrigine 10%
5	48 M	DRESS	Phenobarbitone	9	Phenobarbitone (3+) with 10%, 20%, 30%	Phenytoin (3+) with 10%, 20%, 30% Carbamazepine (2+) with 30% Sodium valproate (3+) with 10%, 30% Lamotrigine (1+) with 30%	-
6	32 F	DRESS	Carbamazepine	6	Carbamazepine (3+) with 10%, 20%, 30%	Sodium valproate (1+) with 10%, Lamotrigine (3+) with 10%, 30% Phenobarbitone (2+) with 10%, 30%	Phenytoin 10%
7	31 M	DRESS	Phenytoin	8	Phenytoin (1+) with 30%	Lamotrigine (1+) with 30%	-
8	26 M	DRESS	Phenytoin	6	Phenytoin (1+) with 30%	-	-
9	63 M	DRESS	Carbamazepine	4	Carbamazepine (2+) with 10%	Lamotrigine (1+) with 30%	-
10	31 M	DRESS	Phenytoin + Phenobarbitone	8	Phenytoin (3+) and Phenobarbitone (3+) with 10%, 20%, 30%	Lamotrigine (1+) with 30%	Sodium valproate 10% and 30%,
11	43 F	DRESS	Phenytoin	4	Phenytoin (2+) with 10%, 30%	Phenobarbitone (2+) with 10%, 30%	Carbamazepine 30%
12	75 F	DRESS	Phenytoin	5	Phenytoin (2+) with 20%, 30%	Phenobarbitone (2+) with 10%, 20%, Sodium valproate (2+) with 10%, 20%, 30%	Phenytoin 10%
13	56 M	DRESS	Phenytoin	1	-	-	Sodium valproate 10%, 20%, 30%
14	60 M	DRESS	Phenytoin	1	-	-	Sodium valproate 10%, 20%, 30%

DRESS: Drug rash with eosinophilia with or without systemic involvement; M: Male; F: Female.

drug eliciting positive patch test reactions in 24%-100% patients with DRESS followed by phenytoin and phenobarbitone in order of frequency^[8,9,11]. The highest patch test positivity was with carbamazepine (50%) followed by phenytoin (33%), phenobarbitone (8.3%) and combination of phenytoin and phenobarbitone in one case and both eliciting positive patch test reactions in this study also corroborate.

Clinical cross reactivity among anticonvulsants occurs frequently from their structural homology. Cross sensitivity between carbamazepine and phenytoin was 18%-50% patients and was as high as 57% in two separate studies^[2,13]. The cross sensitivity from one or more drugs was seen in 11 (92%) of 12 patients with positive patch tests in this study being common in 6 patients having

positivity from carbamazepine. Common cross-reactions were with lamotrigine (4 patients) and sodium valproate (3 patients) and phenobarbitone (2 patients) in order of frequency. Similarly, 3 (75%) of 4 patients with positivity from phenytoin had cross sensitivity to one or more drugs that is phenobarbitone, lamotrigine, and sodium valproate. Another patient with DRESS from phenobarbitone showed positivity to carbamazepine, phenytoin, lamotrigine, and sodium valproate. Although sodium valproate does not cross react with these aromatic anticonvulsants, it was perhaps responsible for positive reaction *per se* in some of the patients in the current study. Nevertheless, multiple drug reactivity is not uncommon and reportedly occurs in 18% patients with DRESS from classes of drugs including anticonvulsants^[14]. The phenomenon is

Table 4 Patch test reactions with different concentrations of drugs

Case No	Clinical diagnosis	Carbamazepine			Phenytoin			Phenobarbitone			Lamotrigine			Sodium valproate		
		10%	20%	30%	10%	20%	30%	10%	20%	30%	10%	20%	30%	10%	20%	30%
1	DRESS	2+	2+	2+	-	-	-	-	-	-	-	-	-	-	1+	1+
2	DRESS	-	3+	3+	-	-	-	-	-	-	-	-	3+	-	-	-
3	DRESS	-	-	1+	-	-	-	-	-	-	1+	1+	1+	2+	-	2+
4	Lichenoid drug eruptions	2+	2+	2+	-	-	-	2+	2+	2+	-	-	-	-	-	-
5	DRESS	-	-	2+	3+	3+	3+	3+	3+	3+	-	-	1+	3+	-	3+
6	DRESS	3+	3+	3+	-	-	-	2+	-	2+	3+	-	3+	1+	-	-
7	DRESS	-	-	-	-	-	1+	-	-	-	-	-	1+	-	-	-
8	DRESS	-	-	-	-	-	1+	-	-	-	-	-	-	-	-	-
9	DRESS	2+	-	-	-	-	-	-	-	-	-	-	1+	-	-	-
10	DRESS	-	-	-	3+	3+	3+	3+	3+	3+	-	-	1+	-	-	-
11	DRESS	-	-	-	2+	-	2+	2+	-	2+	-	-	-	-	-	-
12	DRESS	-	-	-	-	2+	2+	2+	2+	-	-	-	-	2+	2+	2+
13	DRESS	-	-	-	-	-	-	-	-	-	-	-	-	IR	IR	IR
14	DRESS	-	-	-	-	-	-	-	-	-	-	-	-	IR	IR	IR
Total		4	4	6	3	3	6	6	4	5	2	1	7	4	2	4

DRESS: Drug rash with eosinophilia with or without systemic involvement.

considered to be from co-stimulatory signals provided by viral reactivation (herpes family virus reactivation in 76% patients) and/or first-drug sensitization acting as cofactors for enhanced immune response to another drug-protein conjugate. Increased sensitivity/irritability of skin after DRESS, especially when tested too early, is other plausible explanation. Since no patient in the study had received all the anticonvulsants concurrently or sequentially, the multiple positive patch test responses were considered cross-reactions.

It has been recommended to use between 1% and 10% (w/w) of pure drug or 30% (w/w) conc. of the powdered commercial tablet when pure drug form cannot be patch tested^[5]. However, the conc. *per se* was not important in a series of patients with DRESS from carbamazepine for frequency or strength of positive patch test responses over varied drug concentrations from 1% to 20%^[8]. According to Romano *et al.*^[11] anticonvulsants in 20% concentration are sufficient to induce positive patch test results. However, 20% drug concentration elicited only 14 (23%) of 61 positive reactions as compared to 28 (44.4%) positive reactions elicited by 30% drug concentration and 19 (31%) positive reactions from 10% drug concentration particularly in case of carbamazepine, phenytoin and lamotrigine in this study. Whereas, phenobarbitone positivity was more with 10% concentration than higher concentrations while sodium valproate showed equal positivity with both 10% and 30% concentration. Lin *et al.*^[2] also observed similar results with 30% carbamazepine concentration eliciting higher number and more intense positive reactions than 10% concentration. While positive patch test reactions with 5%, 10%, 15% and 20% concentrations of phenobarbitone and carbamazepine occurred in 60% patients, sodium valproate 15%, 30%, 45% and 60% concentrations elicited positivity in one (10%) patient only^[11]. This variability of results is attributed to drugs'

capability to penetrate skin barrier more effectively in higher concentrations and ability to produce its metabolites in the skin in a manner that is dose and the drug type dependent^[15]. Similarly, variability of our results also signifies the need for patch testing with several drug concentrations for accurate results especially when consensus for drug concentration for patch testing in patients with ACDs remains elusive. It is also suggested to use prescribable drug for patch testing for its potential advantage of identifying drug hypersensitivity from excipients itself^[16].

Irritant drug patch test reactions are not uncommon especially with sodium valproate and have been documented even in as low as 1% concentration^[1]. Sodium valproate is highly irritant for being hygroscopic and getting converted rapidly to acidic form. Twenty-four reactions in 9 patients were considered irritant reactions in this study. While all three concentrations of sodium valproate elicited 12 (50%) irritant reactions in this study, carbamazepine, phenobarbitone, phenytoin and lamotrigine produced 4 (16.7%), 3 (12.5%), 3 (12.5%) and 2 (8.4%) irritant reactions, respectively. However, reasons of irritant reactions from drugs other than sodium valproate remain conjectural and might have been from multiple patch test applied concurrently, testing just 4 wk after DRESS (in few cases), or due to constituents of the excipient of prescribable drug that may cause irritant reaction from low pH or positive reactions in already sensitized individuals that may be non-relevant^[17].

Unfortunately, there is little consensus for interval between recovery and time of patch test and interval of 6 wk to 6 mo has been considered appropriate by most workers^[4,9]. The patients who were patch tested within 4-9 mo of recovery in this study had positive drug patch test reactions while longer interval of 10-24 mo elicited no reactions reflecting an important limitation of drug patch testing.

Limitations of the study

Small number of patients and use of commercial drugs for patch testing with possible excipient induced irritant reactions are the main limitations. Timing of one month or ≥ 6 mo after recovery for drug patch testing, patch test drug concentrations, or exposure time might have influenced some results. Late readings at D7 of patch test results were not performed.

In conclusion, drug patch testing appears useful tool to confirm drug imputability established on clinical grounds and cross-reactions in DRESS from anticonvulsants. Carbamazepine was the commonest drug causing positive patch test reactions. Cross-reactions are common among aromatic anticonvulsants and with structurally related lamotrigine while sodium valproate too has potential to cross-react increasing the risk of ACDRs necessitating prudent prescriptions.

COMMENTS

Background

Anticonvulsants, carbamazepine, phenytoin, phenobarbitone (aromatic group), lamotrigine and sodium valproate, are implicated in 20% of all adverse cutaneous reactions (ACDRs) and cross reactions among them are common. It is often difficult to identify the offending drug from temporal correlation/history alone since most patients will be on multiple medications and clinical picture is often not diagnostic. The re-challenge/provocation tests, intradermal tests or skin prick tests are time consuming and require expertise. Moreover, there are ethical concerns due to their ability to re-precipitate severe life-threatening adverse drug reaction such as SJS/TEN. Basophil degranulation/lymphocyte activation tests have limited availability, low sensitivity/specificity and may even be negative during acute stage. Radioallergosorbent test for drug specific IgE, histamine release test, and passive hemagglutination test with sensitivity/specificity nearly similar to skin tests have limited availability/applicability in routine clinical practice. The drug patch test, an *in-vivo* challenge test, in ACDRs is inexpensive, convenient and safe with reasonable certainty. This study evaluated utility of drug patch test for identification of culprit drug as well as cross reactions in patients with ACDRs from anticonvulsants.

Research frontiers

Nearly 95% of adverse drug reactions are Type-A (augmented) reactions which are dose-dependent, predictable from primary and secondary drug pharmacology. Other, Type-B (Bizarre) reactions are idiosyncratic, unpredictable from known drug pharmacology, depend on patient-specific susceptibility factors and manifest varied clinical picture. These can be "non-immune mediated (drug intolerance)" due to inadequate or imperfect metabolic detoxification and present as hemolysis, bone marrow toxicity or neurotoxicity from toxic metabolites, or "pseudo-allergic" due to histamine, leukotrienes or other mediators released from direct basophil/mast cell de-granulation due to drugs like opiates, muscle relaxants or radio contrast media manifesting clinically as asthma, anaphylaxis, and urticaria/angioedema-like reactions. These are often indistinguishable from "true immunologically mediated" immediate (Type- I) hypersensitivity reactions. Depending upon immune effector mechanisms involved the "true immunologically mediated" reactions has four main classes: (1) Type- I or IgE mediated (immediate or anaphylactic/urticaria type); (2) Type- II or complement mediated (cytotoxic); (3) Type-III or immune complex mediated (hypersensitivity vasculitis, serum sickness); and (4) Type-IV or T-cell (CD4 or CD8) mediated (tuberculin or contact dermatitis type) reactions. In Type-IV hypersensitivity reactions activated T-lymphocytes produce different patterns of cytokines and/or cytotoxic factors which are relevant for clinical patterns and drug patch testing: IFN- γ , TNF- α (Th1-Tc1 cells) cause contact dermatitis/tuberculin reaction (type-IVa); IL-4, IL-13, IL-5, eosinophils (Th2 cells) cause maculopapular/exanthematous drug rash and eosinophilia with or without systemic involvement (DRESS) (type-IVb); perforin, granzyme-B, granulysin (cytotoxic T-cells) cause dermatitis, maculopapular drug rash, Stevens-Johnson syndrome (SJS), toxic epidermal necrosis (TEN)

(type-IVc); and CXCL-8, GM-CSF, neutrophils (T-cells) cause acute generalized exanthematous pustulosis (type-IVd). Most positive drug patch test reactions will be elicited in these T-cells mediated ACDRs.

Innovations and breakthroughs

Many studies suggest that diagnosis of drug hypersensitivity by patch testing lacks clarity and standardized definitions of clinical and immunopathological processes. This has resulted in uncertainty that whether patch tests are used appropriately in T-cell-mediated ACDRs. Many studies have also used both skin prick and drug patch tests in different types of ACDRs without ascertaining the immune mechanism relevant to the clinical reaction or the tests used. Failure of carbamazepine to elicit positive patch test responses in some individuals despite T-cell mediated drug hypersensitivity confirmed by positive *in-vitro* T-cell responses also remains poorly understood. There seems no consensus for drug concentration for patch testing in patients with ACDRs. When a commercial form of the drug is used for patch testing it is usual to make up to a 30% by weight conc. of powdered tablet in white soft paraffin. However, there is also evidence that conc is not critical and in a series of patients with DRESS induced by carbamazepine there was no difference in the frequency or strength of positive patch test responses over a range of drug conc from 1%-20%. Thus, it may be better to use 10%, 20% and 30% conc. to avoid missing of true positive results. Usual recommendation is to test with 1%-10% of pure drug and 30% of commercial form. It is advisable to do pre test in healthy controls to avoid conc. high enough that may cause direct toxic, proinflammatory or irritant effects. Opinions also differ whether to use pure drug that is often difficult to procure or prescribable form for patch testing. The later have the advantage of easy availability and diagnosing "drug hypersensitivity" that is actually from the excipient only. There is also little consensus for interval between recovery from ACDRs and time of patch test. An interval of 6 wk to 6 mo has been considered appropriate by most workers. The choice of an appropriate vehicle for antigen preparation is also important. Similarly, late reading of patch test responses at day 7 may be required in some cases. Last but not the least, the role of genetic factors in drug metabolism, drug molecular weight and solubility, and skin barrier function and pathomechanism involved in each type of drug reaction and drug patch test also needs elucidation. More systematic studies and consensual approach in future studies will perhaps resolve some of these issues encouraging wider acceptance of this very safe and important diagnostic test in ACDRs.

Applications

The drug patch test works best for T-cell mediated ACDRs (exanthematous drug eruptions, acute generalized exanthematous pustulosis, DRESS, erythema multiforme major/SJS/TEN, fixed drug eruption and symmetrical drug-related intertriginous/flexural exanthem) particularly from aromatic anticonvulsants and some antibiotics but responses are inconsistent with many other drugs. Further, patch testing in SJS/TEN has low sensitivity. Testing with chemically/pharmacologically similar drugs may also help to identify cross-reactivity for these patients for prudent prescriptions.

Terminology

Erythema multiforme major (EM-major), Stevens-Johnson syndrome (SJS), and Toxic epidermal necrolysis (TEN): This spectral drug hypersensitivity reaction is cytotoxic T-cell mediated and perforin, granzyme-B, granulysin are involved in its immunopathogenesis. Erythema multiforme major is characterized by well defined flat round target-like skin lesions with central necrotic macule/bulla with zone of pallor and outer erythematous rim usually accompanied by mucosal involvement, fever and prostration. It has tendency to become confluent, severe and extensive eventuating to SJS/TEN. SJS is characterized by macular erythema, blisters, and detachment of skin involving 10% body surface area and mucosal ulcerations. Atypical targetoid spots and bullae can occur beyond large sheets of necrotic skin. It may eventuate through SJS-TEN overlap (skin detachment between > 10% and 30% body surface area) to more severe TEN (skin detachment > 30% body surface area) with widespread skin/mucosal detachment and multi-organ involvement often ending fatally; Exanthematous drug eruptions, Acute generalized exanthematous pustulosis (AGEP), and Drug rash with eosinophilia and systemic symptoms (DRESS): This spectral drug hypersensitivity reaction is T-cell mediated and Th2 cell cytokines (IL-4/-13, IL-5) and eosinophils are involved in its immunopathogenesis. Generalized exanthematous drug eruptions is characterized by moderate fever, facial and peri-orbital edema, and prominently

pruritic maculopapular rash that occur during first 2 wk of drug intake (may appear even 10-14 d after stopping it). This can eventuate to AGEF (characterized by non-follicular pustular lesions over face and trunk) or progress to DRESS if multi-organ involvement, lymphadenopathy and eosinophilia develop.

Peer-review

This is an interesting study regarding patch testing and cross sensitivity of adverse cutaneous drug reactions due to anticonvulsants. In general, the methodology of the study is appropriate, the results are significant, and the findings are clinically relevant and scientifically interesting.

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Synergetic role of integrating the departments of cancer registry and clinical research at an academic comprehensive cancer center

McKenzie Bedra, Tammy Vyskocil, Jennifer Emel, Crystal Edwards, Cherif Boutros

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Abstract

Integration of the cancer registry and clinical research departments can have a significant impact on the accreditation process of a Commission on Cancer (CoC) Program. Here in we demonstrate that the integration of both departments will benefit as there is increased knowledge, manpower and crossover in job responsibilities in our CoC-accredited Academic Comprehensive Cancer Center. In our model this integration has led to a more successful cooperative interaction among departments, which has in turn created an enhanced combined effect on overall output and productivity. More manpower for the cancer registry has led to increased caseloads, decreased time from date of first contact to abstraction, quality of data submissions, and timely follow-up of all patients from our reference date for accurate survival analysis along with completeness of data. In 2016, our Annual Facility report showed an additional 163 cases over prediction by the state of Maryland Cancer Registry and a 39% increase in case completeness. As proof of the synergetic effectiveness of our model within one year of its implementation, the cancer center was able to apply for, and was awarded membership from Alliance for Clinical Trials in Oncology, Central IRB, and in turn led to increased clinical trial accrual from 2.8% in 2014 compared to 13.2% currently. Our cancer registry in year one submitted over 150 more cases than predicted, improved quality outcome measures displayed by our Cancer Program Practice Profile reports and had more timely and complete data submissions to national and state registries. This synergetic integration has led to a better understanding, utilization and analysis of data by an integrated team with Clinical Research expertise.

Key words: Cancer registry; Clinical research; Commission on Cancer; Synergetic integration; American College of Surgeons

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Core tip: In the current era, the evolution of healthcare management has focused on limiting resources while increasing the value of healthcare delivery. As hospitals and health care organizations operate under tighter budgets year after year, the executive teams must prioritize and utilize the resources available in the optimal way to produce the best patient care with limited funding. Integrating the cancer registry and clinical research departments can have a significant impact on outcomes. Both departments benefit as there is increased knowledge, manpower and crossover in job responsibilities. This leads to increased caseloads, decreased time from date of first contact to abstraction, and quality and completeness of data.

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MAIN TEXT

Health organizations all over the world are required to set priorities and allocate resources within the constraint of limited funding^[1]. The Commission on Cancer (CoC) is a program of the American College of Surgeons (ACoS) that was established in 1922. CoC membership is composed of 110 individuals who are either surgeons representing the ACoS or who are representatives from one of the 56 national professional organizations or member organizations affiliated with the CoC^[2]. Patients who obtain care at a CoC-accredited cancer program receive many benefits and they are directly related to the quality of their cancer care. They have the opportunity to receive surgical treatment in innovative ways including equipment such as robotic, laparoscopic and other minimally invasive approaches to cancer treatment. Accredited programs participate in multidisciplinary cancer conferences for each specialty where all key physicians are present to decide the best patient-centered care for each individual. In addition to cancer treatment, CoC-accredited programs also offer a vast range of support services for patients who receive treatment at their facilities. Some examples of support services include social work, dietitians, genetics counselors, nurse navigators, nurse practitioners specializing in survivorship which includes life after cancer treatment is complete, clinical research opportunities and a cancer registry that collects data on cancer type, stage, treatment result, and offers lifelong patient follow-up. Being part of a CoC-accredited program raises the bar by ensuring all programs adhere to the

ACoS CoC program standards on an annual basis.

Clinical Research and Cancer Registry departments play an integral role in achieving the standards set forth by the CoC for accredited programs. There is currently one standard for clinical research. CoC Standard 1.9 states, "as appropriate to the cancer program category, the required percentages of patients are accrued to cancer-related clinical research studies each calendar year. The Clinical Research Coordinator documents and reports clinical research study enrollment information to the cancer committee annually^[3]". It is required that each accredited cancer program accrue the minimum percentage of patients based on the program's CoC designated category, and the number of reportable cancer cases on an annual basis. For the cancer registry there are two standards that outline the minimum percentage of follow-up of cancer patient's year around. CoC Standard 5.3 states, "for all eligible analytic cases, an 80% follow-up rate is maintained from the cancer registry reference date". CoC Standard 5.4 states, "a 90 percent follow-up rate is maintained for all eligible analytic cases diagnosed within the last five years or from the cancer registry reference date, whichever is shorter^[3]". These two standards are applicable to both departments as ensuring a high percentage of patients in the cancer registry are followed from the registries reference date forward in turn leads to accurate survival analysis and opportunities for retrospective research.

Each CoC-accredited program is required to report data to the National Cancer Data Base (NCDB) during the annual Call for Data which falls during the beginning of each calendar year. Reporting of data falls under two standards. CoC standard 5.5 states, "each year, complete data for all requested analytic cases are submitted to the NCDB in accordance with the annual Call for Data^[3]". CoC Standard 5.6 states, "annually, cases submitted to the NCDB that were diagnose on January 1, 2003, or later meet the established quality criteria and resubmission deadline specified in the annual Call for Data^[3]". Reporting of this data is mandatory for every CoC-accredited program regardless of the program category on an annual basis. By reporting based on the standards above, it helps measure performance of the program and its cancer care quality. The data is used to monitor treatment patterns and outcomes and to also enhance cancer control and clinical surveillance activities. Utilization of this data helps in the development of effective educational interventions and clinical research to improve cancer prevention, early detection, cancer care delivery and outcomes in health care settings^[3].

Synergetic integration of the cancer registry and clinical research departments can have a significant impact on outcomes of a CoC accredited Academic Comprehensive Cancer Program (ACAD). As the standards of the CoC continue to develop and set the bar higher for accredited programs, individual cancer programs need to meet or exceed these standards. In

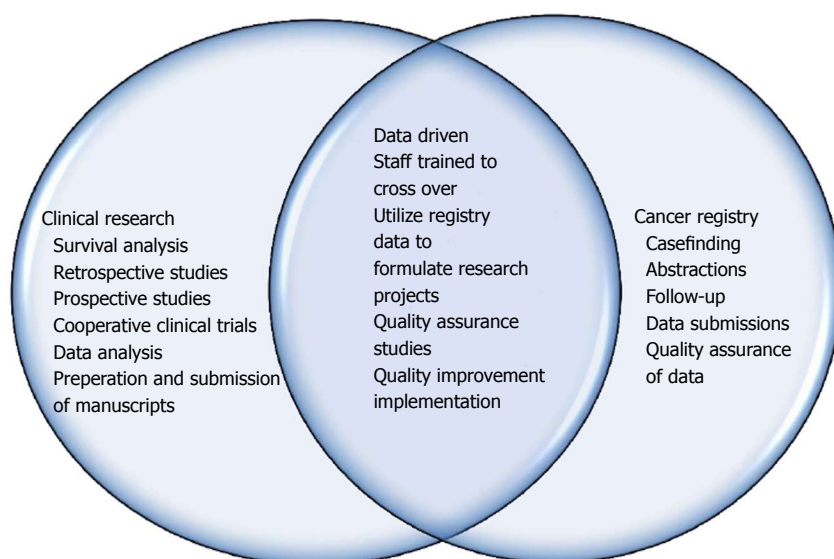


Figure 1 Roles and responsibilities of departments.



Figure 2 Synergistic integration effects on productivity and output.

the current state of healthcare, there is a major question about the priority setting and the dilemma of resource scarcity. This process should be evidenced based and encompass a wide range of challenges^[1]. Today, there is a significant increase in the workload which is needed to comply with CoC accreditation and deliver quality care to patients. As health organizations all over the world are required to set priorities and allocate resources within the constraint of limited funding, this has led to an increase in workload within the cancer registry and clinical research departments^[1]. These departments already have limited resources which has led us to the development of our model to still deliver quality care with the current scarce resources. This project

was started as a vision by our facility to combine two departments which have one common theme, data. The idea was put into place in August of 2015 as there was a need to utilize the vast amount of data available in the cancer registry for research. The two teams were merged and the data was utilized for both departments in many ways.

Results have shown that both departments have benefited as there is increased knowledge, manpower and crossover in job responsibilities. This integration has led to a more successful cooperative interaction among departments, which has in turn created an enhanced combined effect on overall output and productivity. More manpower for the Cancer Registry has led to

increased caseloads, decreased time from date of first contact to abstraction, quality of data submissions, and timely follow-up of all patients from our reference date for accurate survival analysis along with completeness of data. In 2016, our Annual Facility report showed an additional 163 cases over prediction by the state of Maryland Cancer Registry and a 39% increase in case completeness. Figure 1 below shows the roles and responsibilities of the two departments along with how the integration has led to a combined effort and crossover within the departments. Figure 2 below represents the synergistic integration and the flow of effects it has had on our success as an ACAD with less resources and more productivity.

Since becoming a part of Alliance for Clinical Trials in Oncology and Central IRB, our model has led to increased clinical trial accrual from 2.8% in 2014 compared to 13.2% currently. This synergetic integration has led to a better understanding, utilization and

analysis of data by an integrated team with Clinical Research expertise.

Based on our experience, we advocate for synergetic integration and implementation of our model in a CoC-accredited program. Our model will assure the ability to continuously meet standards of accreditation and add value to healthcare delivery while limiting cancer program resources.

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Antioxidants in experimental ischemia-reperfusion injury of the testis: Where are we heading towards?

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Abstract

Testicular torsion (TT) is a medical emergency that

primary affects newborns and young adolescents. It causes testicular injury due to the torsion of the spermatic cord and its components, initially in the venous blood flow and finally in the arterial blood flow. Prompt diagnosis and early surgical management are necessary in managing this urgent situation. The process of the pathophysiological events in ischemia-reperfusion is multifactorial and deals with the perception of the oxidative stress responsible for the consequences of ischemia/reperfusion (I/R) stress following TT. Duration and severity of torsion also play a significant role in the oxidative stress. A detrimental result of the defense system of the testes takes place resulting finally in testicular atrophy and impaired function. Antioxidant factors have been experimentally studied in an effort to front this state. They have been classified as endogenous or exogenous antioxidants. Endogenous antioxidants comprise a structure of enzymic enzymatic and non-enzymic enzymatic particles presented within cytoplasm and numerous other subunits in the cells. Exogenous antioxidants include a variety of natural and pharmaceutical agents that may prevent or ameliorate the harmful effects of I/R injury. In this study we review those factors and their ability to enhance the oxidative status of the testis. A feature insight into where we are heading is attempted.

Key words: Testis; Torsion; Experimental; Ischemia-reperfusion injury; Antioxidants

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Core tip: Testicular torsion is an emergency condition, most commonly seen in newborns and adolescents, which can be considered as an ischemia-reperfusion injury. We provide an overview of the molecular pathogenesis of the disease, and the current evidence of antioxidants use in the experimental torsion-detorsion situation. Possible adaptation of the experimental factors in the clinical practice is discussed.

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INTRODUCTION

Testicular torsion (TT) is one of the most serious surgical emergencies, deriving from the twisting of the spermatic cord and its contents, and causing decreased blood flow to the affected testis and finally testicular atrophy^[1,2]. The testis is exclusively prone to ischemic insults due to anatomical reasons (terminal arteries without anastomoses) and the inflexible properties of the tunica albuginea which restricts satisfactory expansion of the testis^[3]. Although, TT can be detected at any age, it is usually seen during perinatal period and puberty^[4-6]. Two main types of TT exist: Extravaginal and intravaginal^[3]. Extravaginal TT is usually seen during perinatal period, and is ought to the absence of normal fixation between testicular coverings and tunica vaginalis resulting in abnormal motility of the testis within scrotum. Intravaginal TT is most commonly seen in adolescent boys and results from a long mesorchium which allows a greater mobility of the testis within the tunica albuginea^[4].

TT has an annual incidence of about 3.8 per 100000 males less than 18 years^[7], and in cases of bilateral torsion, there is evidence that may be inherited^[8]. If left untreated within 4 to 6 h, loss of spermatogenic cells will occur^[9] leading to harmful results such as infertility and subfertility^[10]. The degree of twisting of the spermatic cord may also play an important role. In animal studies, 720° torsion caused significant reduction blood flow when compared with a twisted spermatic cord of 360° or less^[11].

There are two kinds of injuries responsible for testicular necrosis after TT: The first is related to ischemia (I) injury during torsion, and the second to reperfusion (R) injury during detorsion^[12]. I/R injury can cause cell damage from generation of reactive oxygen species (ROS), proinflammatory cytokines and adhesion molecules, lipid peroxidation, apoptosis, anoxia and alteration in microvascular blood flow, which finally lead to testicular atrophy^[13]. Although the testicular environment is characterized by low oxygen tensions, testes are susceptible to oxidative stress due to the plethora of highly unsaturated fatty acids and the presence of ROS^[14].

Antioxidants represent the first line defense of the organism in order to prevent the harmful consequences of I/R injury occurring in the environment of the testicular cell^[15]. Antioxidants may be classified as endogenous and exogenous^[15]. Endogenous antioxidants include a variety of enzymatic molecules that are

presented within the cytoplasm. Common existing endogenous antioxidant enzymes include superoxide dismutase (SOD), catalase, and peroxidases^[15,16]. Exogenous antioxidants include natural derived components such herb productions^[17-25], vitamins^[26-31], selenium^[32], hormones^[33-36], hormones receptors^[37,38], vascular agents^[39-41], phosphodiesterase inhibitors^[42,43], anesthetic and non-steroid anti-inflammatory drugs^[44-47], mucolytic agents^[48], and hyperbaric oxygen^[49]. All have been used in an effort to prevent the consequences of the oxidative stress in I/R injury.

The aim of this review is to present the pathophysiological changes that take place during I/R injury and to summarize the current literature regarding the role of antioxidants in the prevention of experimental I/R injury. Possible translation from the experimental laboratory studies to clinical practice is discussed.

SEARCH

Literature search

We conducted a search focusing on TT and experimental I/R injury in PubMed publishing over the last five years, between 2012 and 2016. The following search terms were used: "testicular torsion", "experimental ischemia-reperfusion injury", "protective agents". A total number of 22 full papers were extracted.

Pathophysiologic alterations during I/R injury

The pathophysiological alterations during I/R injury are multifactorial and difficult to understand. A cascade of events take place during the course of ischemia and further perturbations of biomolecules in cells are seeing during the blood re-establishment after reperfusion. The basic mechanisms of I/R are described below.

Ischemia injury: The role of Ca²⁺: During ischemia a decrease of cell pH is observed due to accumulation of lactic acid, protons and NAD⁺. To balance these alterations, the cell forces out H⁺ via the Na⁺/H⁺ exchanger system^[50]. Thereafter, Na⁺ ions are swapped for Ca²⁺ by the plasmalemmal Na⁺/Ca²⁺ exchanger, which results in increase of Ca²⁺, exacerbated furthermore during reperfusion. These huge alterations in Ca²⁺ stimulate an array of systems, which finally contribute to cell death^[50-52]. For instance, Ca²⁺ entry into the mitochondria via a mitochondrial protein further increases the lethal concentration of Ca²⁺^[53-55]. In addition, the Ca²⁺ cytosolic elevation during I/R can trigger the Ca²⁺/calmodulin-dependent protein kinases, which further added to cell death and tissue dysfunction^[53]. Additionally, the activation of calpains, a family of cysteine proteases by Ca²⁺ elevation, further degrades a group of intracellular proteins, including cytoskeletal, endoplasmic reticulum, and mitochondrial proteins^[56]. Furthermore, Ca²⁺ forces the creation of calcium pyrophosphate structures and uric acid, a pair that binds to a protein complex called inflammasomes which in turn increase the production

of cytokines IL-1 β , and TNF, which lead to a cytokine cyclone that irritate further the I/R injury^[53].

Reperfusion injury: Studies have shown that during reperfusion, the returned oxygenated blood restores the ATP production but also results in production of ROS, which in turn may modify every biomolecule found in cells, producing further cell dysfunction (oxygen paradox)^[57,58]. Redox molecules derived from nitric oxide (NO), the so called reactive nitrogen species (RNS) interact with ROS and lead to the production of reactive nitric oxide species (RNOS), such as peroxynitrite, responsible for harmful damage of macromolecules, initiation of death of endothelial and parenchymal cells, stimulation and release of pro-inflammatory mediators by various cell groups, and induction of adhesion molecules supporting leukocyte/lymphocyte-endothelial cells interactions, and reduction of protective NO^[57-59].

Oxidative stress: The classic theory of oxidative stress was that it arises from an imbalance between pro-oxidants vs antioxidants intracellular compounds^[39]. Currently, it is believed that oxidative stress is involved in three mechanisms in I/R injury: (1) indirect, through non-radical oxidants such as hydrogen peroxide (H₂O₂); (2) modulator, *via* molecular bond, oxidative or nitrosative modification of principle regulatory proteins; and (3) direct damage by oxidant radicals of DNA, proteins, lipids and carbohydrates^[53,60].

Superoxide anion radical (O₂⁻) is the first product of ROS during I/R injury, and subsequently all the other reactive species are derived from interactions or dismutation with other reactive species^[39]. This is supported by experimental studies showing that I/R were considerably attenuated by treatment with SOD or SOD analogues^[53,61,62]. O₂⁻ oxidizes various biomolecules and inactivates enzymes such as NADH, creatine kinase, and calcineurin^[58]. Sources of O₂⁻ are xanthine oxidoreductase, NADPH oxidase, cytochrome P450, and uncoupled nitric oxide species (NOS)^[53].

Nitric oxide stress: Nitric oxide (NO⁻) is elicited during oxidation of arginine to citrulline, through nitrite or nitrate through the action of xanthine oxidoreductase, or by mitochondrial cytochrome c^[63,64]. NO⁻ plays a protective role in the vascular system by producing dilation of blood vessels, modulating platelets aggregation and adhesions, and inhibiting leukocyte-endothelial adhesive interactions and angiogenesis^[53]. Interactions of NO with O₂ or O₂⁻ forming N₂O₃ or peroxynitrite, are associated with overproduction of NO and O₂⁻ resulting in pathophysiological nitrosative and oxidative stress^[53].

In summary, the oxidative/nitric oxide stress may have negative impact on the cell function in I/R stress through three ways: (1) destruction of cellular macromolecules such as membrane lipids, proteins, and DNA; (2) production of possibly toxic peroxynitrite and other RNOS; and (3) side effects on distinct cellular

systems and functions^[53].

Current antioxidant treatment of I/R injury in experimental TT

Comparable to other tissue-cells which live under aerobic conditions, spermatozoa produce ROS which is a physiological process activity^[65]. Moreover, spermatozoa contain an array of ROS scavengers such as SOD, catalase, and substances such as ascorbic acid, taurine, hypotaurine, albumin, and carnitine to balance any ROS high concentration. However, any increased concentration of toxic metabolic products over the ROS scavenging ability, may cause loss of sperm motility and viability^[66-68].

A substantial number of experimental studies by using different agents have studied experimental TT focusing on the effect of I/R injury on ipsilateral and contralateral testis, on treatment and prevention of this injury^[53]. However, conflicting results are raised due to different animal species, such as rats or pigs, model of I/R injury, age, and technique that has been performed to evaluate the I/R damage^[69]. Furthermore, several experimental studies proposed that the contralateral testis is not affected by unilateral torsion^[70-72]. Nevertheless, there is evidence that both testes are affected, and contralateral testis is not disturbed by initial removal of the torsed testis and pretreatment with antioxidants^[73-75].

There are two therapeutic opportunities to counteract oxidative stress. In the first, the superoxide radical and hydrogen peroxide are eliminated by using specific enzymes such as SOD, catalase, and glutathione peroxidase (GPX) either by administration of these enzymes or by increasing them *in vivo* actions. In the second, radical production is prevented by antioxidant scavenging systems^[66].

Some authors showed that apigenin may prevent lipid peroxidation and protect the antioxidant system^[76,77]. We also found a decrease in immunoreactivity of TNF and IL-10, suggesting a synergistic action of apigenin with endogenous IL-10. This antioxidant effect may be due to the H⁺ donation of the OH⁻ aromatic group^[6]. Among others, we demonstrated^[42] that intraperitoneal injection of erythropoietin and sildenafil protects against I/R injury.

Amlodipine is a calcium channel blocker with antioxidants properties, effectively decreasing experimental vascular ischemia-induced damage in the liver and other tissues^[78]. Dogan *et al.*^[79] examined the effect of amlodipine in a rat model of TT injury. They found a significant decrease of TNF and transforming growth factor-beta in the treatment group, decreases in free radicals and increases in antioxidants such as SOD and GSH.

Goji berry (GB) is a traditional Chinese plant product, from the Solanaceae family with antioxidant effects. In experimental studies, GB has been shown to reduce blood sugar and lipid levels, and exhibits male fertility-enhancing effects, immunomodulating,

antitumor, and anti-fatigue properties. GB is composed from six monosaccharides and influences its effects *via* ion exchange chromatography. In a rat experimental study of TT, administration of GB reduced I/R injury by the antioxidant effects of GB^[9].

Mannitol is usually administered before partial nephrectomy to reduce renal damage due to intravascular volume expansion and its free-radical scavenging^[80]. Kurt *et al*^[81] in a rat model of TT, demonstrated that the treatment with mannitol group had less seminiferous tubules disruptions when compared to the TT group without mannitol treatment.

Hesperidin, is another antioxidant compound belonging to flavones with significant antioxidant effects in many tissues^[82,83]. Hesperidin was given intraperitoneally by Celik *et al*^[12] in an experimental group of rats underwent TT and the sample was compared to control group. They found a reduced effect on histological examinations of the hesperidin group when compared to control, while MDA levels were increased, and SOD, catalase and GSH levels were decreased as compared to the control group, concluding that hesperidin has positive results in cases of TT.

Polyphenolic catechins are components of green tea and comprise (-)- epicatechin, (-)- epigallocatechin, (-)- epicatechin gallate, and (-)- epigallocatechin gallate (EGCG)^[84]. Sugiyama *et al*^[85] studied an experimental rat model by producing 4 hours' ischemia and giving orally a single dose of (-)- EGCG 1 h before reperfusion. Histologic examination 4 wk after reperfusion found that EGCG protected against testicular damage from I/R injury and inhibited a further decrease in the activity of SOD.

Dexketoprofen, is a racemic mixture from the arylpropionic acid family of NSAIDS. Yildirim *et al*^[86] studied the intraperitoneal effect of dexketoprofen in a rat model of I/R injury. Malondialdehyde (MDA) levels were investigated in tissue and serum of torsioned testicles in the dexketoprofen group and control group. They found a statistically lower serum MDA levels in the dexketoprofen group compared to control group, and decreased, but not statistically significant, pathological changes in the spermatogenic cells of the control group.

Tyrphostin AG 556 is a tyrosine kinase inhibitor and belongs to the tyrphostin group which has been assessed in animal models of spinal cord and coronary I/R injury^[87,88]. Karaguzel *et al*^[89] investigated the effect of Tyrphostin AG 556 by giving it intraperitoneally and measured the following biochemical parameters: MDA, ischemia modified albumin, signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1), epidermal growth factor like domain-containing protein1, oxidative stress index, total oxidant status, and total antioxidant status. They concluded that tyrphostin AG 556 has a protective effect on I/R injury

The protective effect of udenafil citrate, piracetam and dexmedetomidine in different doses was evaluated by Tuglu *et al*^[90] and found that all these agents have antioxidant effects on I/R injury.

Grape seed proanthocyanidin extract has been reported to display better antioxidant activity than other antioxidants such as vitamin C, vitamin E, and gallic acid^[91]. Bayatli *et al*^[92] examined the protective effect of grape seed proanthocyanidin after TT performed for 2 h and administered it daily for a week prior to torsion/detorsion. They reported that grape seed proanthocyanidine prohibited the rise of MDA, apoptosis and endothelial nitric oxide synthase expression and enhanced testicular morphology.

Carnosine, is a dipeptide found in high amounts in mammalian tissues^[93]. Abbasoglu *et al*^[94] demonstrated that carnosine treatment has a protective effect on pro-oxidant and antioxidant status in rat testes with I/R injury.

Ozone has been studied as a potential therapeutic agent for the treatment of various physio-pathologic conditions expressing high levels of ROS^[95,96]. Ekici *et al*^[97] assessed the potential effects of ozone in testicular function and morphology in a rat experimental study, in a mixture of ozone/oxygen and compared the results with those of melatonin. They found similar results in the amelioration of I/R injury between melatonin and ozone, but in different pathways.

Ethyl pyruvate, a ROS scavenger, has been found to ameliorate in different conditions such as sepsis, acute pancreatitis, burn, radiation injury and hemorrhagic shock^[98,99]. Turkmen *et al*^[100] reported that ethyl pyruvate has a positive effect on torsion-detorsion associated I/R injury in an experimental rat model.

Carvedilol is a third generation vasodilator agent which has been used in the treatment of hypertension, congestive heart failure and ischemic heart disease^[101,102]. Parlaktas *et al*^[103] investigated the antioxidant effects of carvedilol against I/R injury, and found a decrease in MDA and protein carbonyl and an increase in the level of antioxidant enzymes SOD, and GPX, but not histopathological changes against the control group. They concluded, that carvedilol may have a potential therapeutic value and improve fertility in the clinical practice in patients with TT.

Jiang *et al*^[104] investigated the effect of intraperitoneally injected hydrogen rich saline solution on the protection against testicular damage induced by I/R injury in rats. They found a significant decrease of MDA and a significant improvement of SOD activity in the group of rats which received hydrogen rich saline solution. Therefore, hydrogen rich saline solution may have a protective and therapeutic action against testicular damage.

Inhaled hydrogen gas has been shown to produce a therapeutic activity in a middle cerebral artery occlusion in a rat model and reduce infarct volumes of brain, liver, and myocardium^[105,106]. Lee *et al*^[107] studied the possible therapeutic properties of inhaled 2% hydrogen in pubertal rat model underwent testicular I/R injury. The results of histopathological and biochemical studies suggested that inhalation of hydrogen gas has anti-apoptotic and anti-oxidant properties in cases of TT.

Alpha-lipoic acid is an eight-carbon endogenous cofactor which works against oxygen radicals^[108]. It is established that α -lipoic acid catches hydroxyl and nitric oxide radicals, peroxynitrite anions and hydrogen peroxide. Moreover, α -lipoic acid may act indirectly by enhancing the level of other natural antioxidants such as glutathione, ascorbic acid and tocopherol^[31,109-111]. Ozbal *et al.*^[108] investigated the role of α -lipoic acid in testicular I/R injury in rats and concluded that it is a potential beneficial agent in preserving testicular function.

Genistein is an isoflavone extracted by soy^[112] which displays anti-oxidant and anti-inflammatory properties^[113]. Furthermore, genistein promotes steroidogenesis by restriction progesterone synthesis and decreases secretion of cortisol and corticosterone in mature female pigs^[114]. In addition, it has a protective role against gamma irradiation-induced testicular dysfunction^[115]. Recently, Al-Maghrebi *et al.*^[116] reported that genistein protects the extracellular matrix of the testis which is responsible for the structural integrity of the testicular components, and prevents spermatogenesis's suppression, mitigating oxidative stress and apoptosis in experimental testicular I/R injury.

Nuclear factor kappa B plays a crucial role in immune response, cellular proliferation, inflammatory, and apoptosis^[117]. Pyrrolidine dithiocarbamate (PDTC) is a stable low-molecular thiol compound which acts by neutralizing ROS^[118]. Kemahli *et al.*^[118] studied the antioxidant effect of PDTC in a TT model and found that administration of PDTC exaggerates the antioxidant system by lowering MDA levels, increasing SOD activity and improving Johnson scores of biopsy specimen.

Urocortin, is a 40-amino acid peptide found in different organs, such as digestive tract, cardiovascular and reproductive system^[119]. For instance, urocortin has been shown that protect cardiovascular system against I/R injury^[120]. Sumii *et al.*^[121] investigated the role of urocortin in testicular apoptosis in an experimental I/R rat model and found a cytoprotective role in germ cells through the activation of anti-apoptotic proteins.

Melatonin is an endogenous compound secreted by the pineal gland and influences reproduction *via* its activity on the hypothalamus^[122]. Kurcer *et al.*^[123] reported that melatonin protects testicular tissue against oxidation and alleviates histopathologic changes after experimental testicular I/R injury. Metformin belongs to the biguanide family and has the capacity to reduce ROS^[124]. Asghari *et al.*^[125] investigated a combined use of melatonin and metformin in a rat model and found that may protect the testes from I/R injury by restoring SOD activity, and MDA and myeloperoxidase levels.

Very recently Erol *et al.*^[126] investigated the effect of a antioxidant factors combination, constituting either by L-carnitine, fructose, citric acid, ascorbic acid, cyanocobalamin, selenium, coenzyme Q10, zinc and folic acid or fructose, cellulose microcrystalline, pygeum shell, L-arginine, L-carnitine, zinc, vitamin E, folic acid, vitamin B6, sodium selenite, and hydroxypropyl methyl cellulose. They found that combined antioxidants were

more effective than one protective antioxidant by reducing apoptosis and preventing I/R injury.

Antioxidants and I/R injury in clinical practice

The large body of experimental studies demonstrated undoubtedly that oxidative stress is a dominant factor in the creation of testis impairment after I/R injury. Furthermore, all these antioxidative compounds have been sought to be clearly capable to protect testicular function from oxidative stress. However the relationship between experimental results and clinical practice has not come together until now. A feature mandatory pursuit is to advance understanding of the basic mechanism of oxidative stress in the male reproductive tract and to develop optimizing antioxidant factors in order to treat the pathological consequences from imbalance in the oxidation state of testicular tissue. These mandatory demands are beyond laboratory ways that outline the present approach to counterbalance the deleterious effects of TT.

CONCLUSION

Currently, a large number of studies investigate the role of I/R injury in experimental animal models and many antioxidants and free radical scavengers have been studied to indicate their possible application in human beings. However, the molecular mechanism by which these agents may control the harmful effect of TT has to be clarified. Moreover, experimentally checked drugs or compounds still anticipate clinical utilization. Additional experimental and future clinical studies have to be performed to further assess the effects on antioxidant therapy.

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Role of metabolic stress for enhancing muscle adaptations: Practical applications

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Abstract

Metabolic stress is a physiological process that occurs during exercise in response to low energy that leads to metabolite accumulation [lactate, phosphate inorganic (Pi) and ions of hydrogen (H⁺)] in muscle cells. Traditional exercise protocol (*i.e.*, Resistance training) has an important impact on the increase of metabolite accumulation, which influences hormonal release, hypoxia, reactive oxygen species (ROS) production and cell swelling. Changes in acute exercise routines, such as intensity, volume and rest between sets, are determinants for the magnitude of metabolic stress, furthermore, different types of training, such as low-intensity resistance training plus blood flow restriction and high intensity interval training, could be used to maximize metabolic stress during exercise. Thus, the objective of this review is to describe practical applications that induce metabolic stress and the potential effects of metabolic stress to increase systemic hormonal release, hypoxia, ROS production, cell swelling and muscle adaptations.

Key words: Metabolic stress; Muscle mass; Exercise

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Core tip: This review aimed to describe practical applications for inducing metabolic stress and the potential effects on the increase of systemic hormonal release, hypoxia, reactive oxygen species production, and cell swelling. These effects are responsible for enhancing muscle adaptations through changes in exercise routines (intensity, volume, rest between sets) and training modes (resistance training, low-intensity resistance training plus blood flow restriction, and high intensity interval training).

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INTRODUCTION

It has been reported that chronic exercise can promote changes in many organs because of cellular adaptations. Skeletal muscle is extremely adjustable in response to contractile activity^[1,2], therefore, repeated muscle contractions during exercise can lead to numerous metabolic modifications^[3,4]. Overtime, these adaptive responses have shown beneficial effects on health, body composition and performance^[5-7].

During acute exercise, the energy used by skeletal muscle contractions are essential in transforming organelles, enzymatic activity, intracellular signaling and transcriptional responses^[8-10]. Metabolic stress is a physiological process that occurs during exercise in response to low energy which leads to metabolite accumulation [lactate, phosphate inorganic (Pi) and ions of hydrogen (H⁺)] in muscle cells^[11,12]. Researchers have suggested that metabolic stress has an important impact on hormonal release, hypoxia, cell swelling and production of reactive oxygen species (ROS)^[13-15]. All of these components can initiate anabolic signaling for muscle growth and adaptations on energy metabolism^[16].

In situations with elevated ATP hydrolysis and glycolytic flux in muscle cells, there is a great accumulation of adenosine monophosphate (AMP) and metabolites^[12,17,18]. Furthermore, the reduction of intracellular oxygen levels can also lead to hypoxia^[19]. All these metabolic parameters are a powerful stimulus to activate AMP-activated protein kinase (AMPK) and hypoxia-inducible factor (HIF-1 α) pathway, the main regulators of mitochondrial biogenesis and angiogenesis^[20,21].

Moreover, metabolite accumulation and hypoxia that is produced during exercise may increase ROS production through mitochondrial electron transport chain^[22,23]. It is well established that ROS production by endurance exercise has positive effects on mitochondrial adaptations because it stimulates peroxisome proliferator-activated receptor gamma coactivator (PGC-1 α) and p38 mitogen-activated protein kinase (p38-MAPK) pathways^[24]. Scientific evidence shows that suppression of ROS production through the use of the antioxidants impairs some adaptive responses to endurance exercise^[25,26], and these results suggest that ROS production has positive effects on mitochondrial adaptations.

Nevertheless, besides stimulating mitochondrial biogenesis and angiogenesis, the metabolic stress also has positive effects on muscle hypertrophy. Resistance training (RT) has great impact on increasing metabolite accumulation, which influences hormonal

release, hypoxia, ROS production and cell swelling. All these processes can mediate anabolic signaling that stimulates muscle protein synthesis and activation of satellite cells^[13-15].

In this context, changes in acute exercise routines (intensity, volume and rest between sets) are the main factors in determining the magnitude of metabolic stress^[27-29]. Furthermore, blood flow restriction training has been considered a tool to maximize metabolic stress^[30,31]. Studies have reported great effects of this training method on aerobic adaptations and muscle hypertrophy^[32,33].

Therefore, the purpose of this paper is to describe practical applications that cause metabolic stress. In addition, we will discuss the potential effects of metabolic stress on the increase of systemic hormonal release, hypoxia, ROS production, and cell swelling for enhancing muscle adaptations.

RESISTANCE TRAINING

Skeletal muscle hypertrophy depends on positive muscle protein balance (protein synthesis exceeds breakdown)^[34]. Thus, RT is excellent for the stimulation of anabolic signaling and the promotion of muscle hypertrophy^[35]. Metabolic stress is one of the primary mechanisms that makes RT increase muscle mass, mainly due to the rise of anabolic hormonal release, hypoxia, ROS production and cell swelling^[13]. However, studies have shown that the magnitude of metabolic stress depends on the changes of acute RT program variables^[14,15].

Scientific evidence shows that load, number of repetitions, and rest between intervals are important factors to induce metabolite accumulation. Gonzalez *et al*^[29] found that acute RT with moderate repetitions combined with short rest intervals (70% 1RM, 10-12 repetitions and one minute rest interval) shows an increase in blood lactate, serum concentration of lactate dehydrogenase, growth hormone (GH) and cortisol when compared to higher loads, low repetitions combined with longer rest intervals (90% 1RM, 3-5 repetitions and three minute rest intervals). Concerning these findings, duration of rest intervals may reflect directly on the magnitude of metabolic stress. In a review study, researchers demonstrated that short interval sets (less than one minute) are essential in increasing blood lactate and GH production, mainly because of insufficient recovery of phosphocreatine and H⁺ accumulation^[36].

Additionally, Nishimura *et al*^[37] demonstrated higher effects of muscle hypertrophy when RT is performed during hypoxia, possibly because of the strong influence of hormonal release, the recruitment of fast-twitch muscle fibers, ROS production and cell swelling^[38]. During RT, muscle contractions compress blood vessels in active muscles, and this occlusion can lead to a reduction of oxygen levels and, consequently, resulting

in a hypoxic environment^[39]. Intramuscular hypoxia during exercise can increase the necessity of anaerobic latic metabolism by activation of HIF-1 α that regulates the expression of glycolytic enzymes^[40]. Thus, exercise that produces high levels of lactate can be associated with hypoxia. One study showed that performing hypertrophy-type RT (70% 1RM, 10 repetitions and 90 s rest intervals) induces higher production of lactate and reduction in pH than performing a strength-type RT (85% 1RM, 4-6 repetitions with five minute rest intervals)^[41]. In this context, it can be hypothesized that RT can generate hypoxia when performed at moderate/high repetitions combined with short rest intervals, possibly due to a high demand on anaerobic metabolism.

Furthermore, another study found that knee extension RT at low intensity (50% 1RM) generates a significant decrease in muscle oxygenation when compared to high-intensity (80% 1RM) exercise performed with one-second rest between repetitions^[42]. These findings suggest, keeping continuous tension on muscles without relaxation can be essential to reducing oxygen levels and maximizing the levels of hypoxia in the skeletal muscle.

Research suggests that ROS production also has important implications on muscle hypertrophy^[43,44]. In addition, studies have shown that utilization of antioxidants can modify protein signaling after a RT session and impairs muscle mass gains^[45,46]. Muscle contractions during exercise produces ROS at low physiological levels and plays an important role in cell signaling to promote beneficial adaptations^[47]. Researchers have found that the production of ROS has an influence in stimulating anabolic signaling, because ROS can act with a signaling molecule to activate the mammalian target of rapamycin (mTOR) through IGF-1 and MAPK pathways^[48,49].

Although it is becoming clear that ROS has a profound impact on muscle hypertrophy, the limits of these adaptations are not clear. Hornberger *et al.*^[50] observed that selenium-deficient transgenic mice (animals with decreased protein expression of antioxidant enzymes containing selenium) exhibited an increased muscle hypertrophy when stimulated by synergist ablation (a muscle overload model), compared to other animals. In this study, rapamycin treatment (a pharmacological inhibitor of mTOR) completely abolished the hypertrophy effect, thus proving that mTOR is necessary for hypertrophy. It is interesting to note that, contrary to this study (where muscle antioxidant defense was decreased and muscle hypertrophy was optimized), other studies evaluating the impact of antioxidants in humans (through vitamin E and C supplementation) were shown to impair muscle hypertrophy response and cell signaling leading to muscle hypertrophy^[45,46]. Several studies have observed that RT increases hypoxia, metabolite accumulation and ROS production, which seems to be strictly related^[22,23,51,52]. In this context, we

can hypothesize that RT with moderate/high repetitions and short rest intervals can be a stimulus to produce ROS.

Another potent anabolic signaling event produced by RT is cell swelling. Studies have demonstrated that cell swelling mediated by hydration can lead to an increase in protein synthesis and a decrease in proteolysis mainly through the activation of MAPK pathway^[53-55]. During intense muscle contractions, veins are obstructed but the arterial system keeps the delivery of blood active^[13]. This process can increase intracellular swelling, which leads to an increased pressure against the cytoskeleton. Thus, the cell perceives a threat and initiates an anabolic signaling response to promote reinforcement of its ultrastructure^[56]. Studies indicate that cell swelling occurs during metabolite accumulation (lactate, H⁺ and Pi) which leads to additional intracellular fluid^[57,58]. Therefore, it seems reasonable to conclude that RT during hypertrophy causes high metabolite accumulation and can promote more cell swelling than strength RT.

Finally, another aspect that we should consider, especially among well-trained subjects, is RT with moderate/high repetitions until failure. Recent studies show that, when RT is executed with low load (30%-50% 1RM and 25-35 repetitions) until failure, hypertrophy is similar when compared to high load (70%-90% 1RM and 8-12 repetitions)^[59-61]. Although no studies have confirmed this hypothesis, we believe that muscular failure can exert additional metabolic stress and then induce anabolic signaling. These findings suggest that the greater time under tension with moderate/high repetitions without relaxation combined with short rest interval and muscular failure can generate a strong hypertrophic response similar to RT with high loads. However, caution should be taken, because restricting rest periods would cause a reduction in the volume performed during a RT session, thus affecting hypertrophy process negatively^[62].

This effect can be caused by high metabolic stress, leading to anabolic signaling through hypoxia, hormonal release, ROS production and cell swelling (Figure 1).

LOW-INTENSITY RESISTANCE TRAINING PLUS BLOOD FLOW RESTRICTION

During the last decade, blood flow restriction training (BFRT), also known as KAATSU or occlusion^[63], combined with low-intensity strength training (20%-30% 1RM), has been shown to increase strength and muscle size among trained/untrained athletes^[64-66] injured^[67] and the elderly^[68]. This training model requires the use of cuffs that are placed at the proximal ends of the upper arms or thighs reducing blood flow from the muscle (approximately 100-200 mmHg). Thus, the external pressure applied maintains arterial inflow while blocking venous outflow of blood^[69], resulting in an ischemic/hypoxic environment that enhances the training effect^[70].

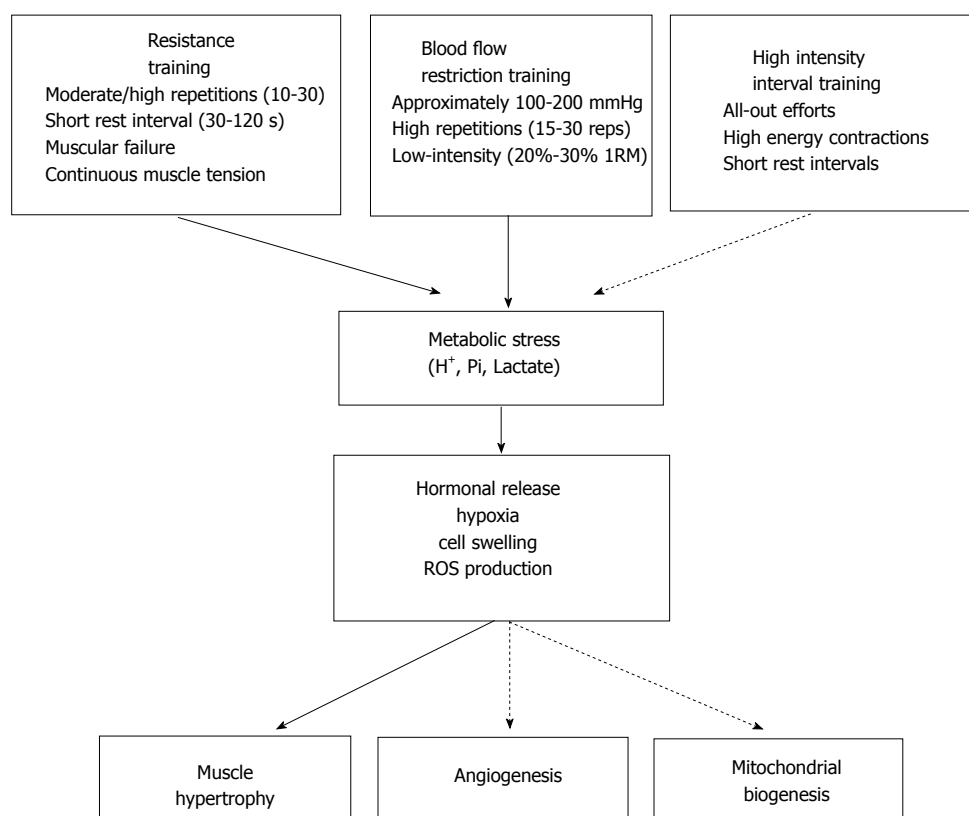


Figure 1 Role of metabolic stress induced by different kinds of training (resistance, blood flow restriction and high intensity interval intraining) for enhancing muscle adaptations. ROS: Reactive oxygen species.

Several studies have compared low-intensity strength training with BFRT and high-intensity without BFRT and demonstrated a significant increase in muscle cross-section area in both exercise protocols^[64,69,71,72]. However, RT performed with moderate/high intensities seems to lead to similar degrees of muscle hypertrophy when combined with BFRT. It is not clear if the maximal degree of muscle hypertrophy can be optimized by increasing external loads or if the ceiling for maximal hypertrophy is achieved with low-moderate loads^[73].

Cumming *et al.*^[74] performed a study with nine healthy volunteers performing five sets of unilateral knee extension at 30% of 1RM until failure combined with BFRT and the same workout without BFRT. Analysis of muscle biopsies revealed a rapid translocation of heat-shock proteins (HSP27 and α B-crystallin) from cytosol to cytoskeletal structures, both of which have been identified as important HSPs for repair and stabilization of stressed and damaged proteins^[75]. This indicates that cytoskeletal proteins are stressed during BFRT even without myofibrillar disruptions. Thus, muscle hypertrophy induced by BFRT seems to be mediated by metabolic stress and mechanical tension, and sarcolemmal-bound mechanosensors (*i.e.*, integrins) stimulate intracellular anabolic and catabolic pathways, which convert mechanical energy into chemical signals, promoting protein synthesis instead of degradation^[76].

Suga *et al.*^[77] investigated metabolic stress (intramuscular phosphocreatine (PCr), Pi, Diprotonated phosphate-H₂PO₄ and Intramuscular pH) in subjects that performed four unilateral plantar flexion (two min of 30 repetitions/min) using three different intensities (20%, 30% and 40% 1RM) with two resistance exercises (20% 1 RM and 65% 1RM) without BFRT. They concluded that 30% of 1RM induced a similar intramuscular metabolites and pH response than high-intensity RT without BFRT. In addition, Suga *et al.*^[31] also showed that multiple low-intensity BFRT sets increase fast-twitch fiber recruitment that could assist the slow twitch fiber to keep the strength during training, however, the authors did not observe statistical significance between multiple sets of high intensity exercise without BFRT. Therefore, these results suggest that multiple-set exercise are more effective than single-set RT.

Previous studies have shown that metabolic stress induced by low-intensity plus BFRT increases GH secretion and muscle hypertrophy^[64,65,78], furthermore, this could stimulate metabolic stress markers, such as IL-6^[79,80]. The recovery process is initiated by IL-6 by modulating muscle regulatory genes (*i.e.*, MyoD)^[81-83] and activating muscle satellite cells^[80], and therefore may play a role in regulating muscle growth/hypertrophy^[80].

An acute increase in anabolic hormones (*e.g.*, testosterone, GH) has been found during short rest periods (30 to 60 s)^[84], however, regarding cytokine production,

a recent study compared 30 s vs 90 s of rest after four sets of squat and four sets of bench press with 70% of 1RM until failure without BFRT in healthy adults and observed higher IL-6 levels when 90 s rest was used^[85]. In addition, Phillips *et al.*^[86] reported greater post-exercise IL-6 concentrations with 65% of 1RM compared to 85% of 1RM with two minutes of recovery. Thus, short rest period induce an acute increase in anabolic hormones, however, it seems that longer recovery intervals combined with higher loads contribute to an increase in IL-6 concentration during RT.

Therefore, changes in variables, such as recovery intervals, volume, intensity, and repetition speed, could be used to optimize the specific adaptation during low-intensity RT plus BFRT.

HIGH-INTENSITY INTERVAL TRAINING

Studies have investigated the benefits of metabolic stress on skeletal muscle remodeling, angiogenesis, mitochondrial biogenesis, performance, and high-intensity interval training (HIIT) has shown to be a promising training routine. This exercise/training routine is based on high-intensity exercise sets with passive or low-intensity intervals between them. Endurance training adaptations have been found with HIIT^[87,88].

The HIIT configuration allows intervals of effort and pause, and the various forms of stimuli can cause adaptations, such as: (1) mechanical stretching and muscle tension; (2) increase of ROS; (3) increase of intramuscular calcium concentrations and (4) changes of energy "status" in the cell.

Two HIIT routines that are commonly used are: four sets of 30 s at 100%^[88] and four sets of four minutes^[89] at 90%-95% of the maximum power (Pmax), velocity (Vmax) or maximum heart rate (HR max). Wahl *et al.*^[90] compared the acute responses of these two routine with another routine done continuously (two hours at 55% Pmax) in triathlon athletes and found that the most intense stimulus (four sets of 30 s at 100%) generated higher metabolic acidosis (pH) and higher concentrations of anabolic hormones (testosterone and GH) after the session. Supporting these results, Wahl *et al.*^[91] compared the use of buffer solution (sodium bicarbonate) and placebo with HIIT (four sets of 30 s at 100%), and showed a significant decrease in pH in the placebo group with increases in GH compared to the buffer group. The elevation of these hormones mean hypertrophic adaptations and also important stimuli expression of oxidative enzymes and erythropoiesis, promoting improvements in aerobic performance. This can be explained by the direct stimulation of bone marrow by testosterone, supporting the synthesis of erythropoietin in kidney cells^[92].

Mitochondrial biogenesis is another adaptation of great importance in this process and one of the most studied. A key molecule for this adaptation is PGC-1 α , a coactivator of several transcription factors

related to metabolic and mitochondrial adaptations^[93]. Burgomaster *et al.*^[87] found that six weeks of HIIT (three times per week, four to six sets of 30 at 100%) and continuous training (five times per week, 40 to 60 min at 55% VO_{2max}) showed significant improvements in mitochondrial functions with optimization lipid oxidation, increased activity of oxidative enzymes (citrate synthase and 3-hydroxyacyl CoA dehydrogenase) and contents of PGC-1 α . The important finding of this study was the difference in the duration of training sessions, ranging from approximately 1.5 h to 4.5 h per week for HIIT and continuous training, respectively.

Due to the importance of PGC-1 α , the expression and activation of proteins that stimulate it has great relevance. Two proteins, which are unquestionably stimulated by metabolic stress, are p38MAPK and AMPK^[94-96]. Gibala *et al.*^[97] showed a significant increase in phosphorylation of AMPK and p38MAPK after acute sessions of HIIT (four sets of 30 s at 100%), and despite a great increase in mRNA of PGC-1 α , its protein content did not change. Additionally, Little *et al.*^[98], using the same protocol of exercises, showed significantly higher values of p38MAPK after exercise, as well as an increase of 750% of mRNA PGC-1 α and 66% of protein already in the nucleus of muscle cells, confirming the potential of these training routine.

Mitochondrial biogenesis and angiogenesis are essential for aerobic adaptations and improvement of performance. Considering the efficiency of HIIT (short training repetitions and metabolic stress), with BFRT seems to be beneficial to increase vascular adaptations. Consequently, Taylor *et al.*^[32] compared acute HIIT (four sets of 30 s at 100%), with HIIT + BFR (cuff in the thigh, two minutes, 130 mmHg). The results of these biopsies (vastus lateralis) showed a significant increase in p38MAPK after HIIT and HIIT+BFRT, with no differences between them. After three hours of exercise, a significant increase in mRNA PGC-1 α was observed, vascular endothelial growth factor (VEGF) and its receptor (VEGFR-2), however mRNA of HIF-1 α only increased in HIIT + BFRT. These results indicate that HIIT by itself is capable of stimulating angiogenesis, but the fact that only HIIT + BFRT increased HIF-1 α cannot be overlooked, because it is a key factor for hypoxia and metabolic stress. Low PO₂ increases concentrations, favoring translocation to the nucleus and subsequent activation of VEGF in the human skeletal muscle^[99].

CONCLUSION

Changes in acute exercise routine variables, such as intensity, volume, recovery interval and type of training are determinants that influence the magnitude of metabolic stress. Despite, traditional training protocol, such as RT, increase metabolite accumulation and influence hormonal release, hypoxia, ROS production and cell swelling. In this review, we discussed that low-intensity RT plus BFRT and HIIT are alternative exercise

routines that increase metabolic stress and muscle adaptation among different populations. However, the difference between exercise protocols used in literature and different levels of physical fitness should be considered when interpreting the results.

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Targeted temperature management in neurological intensive care unit

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most promising neuroprotective therapy against hypoxic/ischemic encephalopathy (HIE). In addition, TTM is also useful for treatment of elevated intracranial pressure (ICP). HIE and elevated ICP are common catastrophic conditions in patients admitted in Neurologic intensive care unit (ICU). The most common cause of HIE is cardiac arrest. Randomized control trials demonstrate clinical benefits of TTM in patients with post-cardiac arrest. Although clinical benefit of ICP control by TTM in some specific critical condition, for an example in traumatic brain injury, is still controversial, efficacy of ICP control by TTM is confirmed by both *in vivo* and *in vitro* studies. Several methods of TTM have been reported in the literature. TTM can apply to various clinical conditions associated with hypoxic/ischemic brain injury and elevated ICP in Neurologic ICU.

Key words: Targeted temperature management; Neuroprotective therapy; Ischemic/hypoxic encephalopathy; Intracranial pressure; Surface cooling; Endovascular cooling

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Core tip: Two main purposes of targeted temperature management (TTM) in patients admitted in neurological intensive care unit are neuroprotective therapy and intracranial pressure (ICP) control. TTM is the most potent neuroprotective treatment due to its numerous methods of protection against ischemic/hypoxic injury. TTM provides capable ICP reductive action. Two most popular methods using in clinical practice and clinical trials are invasive endovascular technique and non-invasive surface cooling. Fast induction, smooth maintenance and slow rewarming are the important steps to achieve ideal TTM.

Abstract

Targeted temperature management (TTM) shows the

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INTRODUCTION

Clinical benefit of therapeutic hypothermia in patients with post-cardiac arrest syndrome (PCAS) has been demonstrated by two randomized control trials since 2002^[1,2]. However, the term "therapeutic hypothermia" has been replaced with "targeted temperature management (TTM)" since 2011 after the meeting of five major professional physician societies^[3]. TTM defines as a type of treatment that reduces a subject's core temperature until a specific target with the purpose in salvage or alleviate the tissue injury from deficiency of blood perfusion^[4]. TTM is recognized as a only established neuroprotective therapy for hypoxic/ischemic brain injury, particularly in patients after cardiac arrest^[5]. The clinical practice guidelines state that TTM should apply as a major treatment for patients following successful resuscitation from cardiac arrest^[6-10].

Elevated intracranial pressure (ICP) is one of the common conditions found in patients admitted in neurologic intensive care unit (ICU)^[11]. Many clinical and animal trials demonstrate that TTM effectively lowers ICP^[12]. However, the application of TTM as ICP control in each particular disease, for examples in primary intracerebral hemorrhage (ICH), subarachnoid hemorrhage (SAH), traumatic brain injury (TBI) and cerebral infarct, needs to be proved by large randomized controlled trial^[13].

HYPOXIC/ISCHEMIC CASCADE

Hypoxic/ischemic brain damage is associated with the abruptness of cerebral blood flow (CBF)^[14]. Cessation of brain circulation leads to compound neurologic damages, the so-called hypoxic/ischemic cascade^[15]. After the deficiency of oxygen and circulation supplying occur, adenosine triphosphate (ATP) manufacturing malfunction develops^[16]. Neurons and glials change from aerobic to anaerobic process, resulting in accumulation of lactic acids^[17]. Cells become depolarized due to the sodium-potassium ATPase pumps failure, letting ions, particularly calcium (Ca^{2+}), to invade themselves^[18]. Elevated intracellular Ca^{2+} stimulates the release of the well-known excitatory amino acid neurotransmitter such as glutamate^[19]. Glutamate permits further Ca^{2+} influx the cells by activate the opening of Calcium-permeable NMDA receptors and AMPA receptors^[20]. After excessive calcium Ca^{2+} influx, the production of deleterious substances including various free radicals, reactive oxygen species, phospholipases, ATPases, and endonucleases, the so-called excitotoxicity materials, initiates^[21]. Membrane and mitochondria break down and lead to development of necrotic cells and apoptosis. Glutamate and other harmful materials are then released

from these necrotic cells into the environment^[22]. These materials cause further damage to adjacent cells. This continuous injury, the so-called reperfusion damage, usually starts when the cerebral tissue gets reperfused^[23]. Inflammatory scavengers get accumulated to eat up the debris tissue and then generate many cytokines^[24]. These toxic materials disrupt the blood-brain barrier (BBB). Destroyed BBB conducts to leakage of huge protein molecules particularly albumins into the environment causing brain edema^[25]. Brain edema produces pressure effect of and further harm to adjacent brain tissue^[26]. The hypoxic/ischemic cascade are shown in Figure 1.

ICP

The theory of ICP, the so-called Monro-Kellie doctrine, was first postulated by Alexander Monro in 1783 before George Kellie published the article support Monro's idea in 1824^[27,28]. This theory states that since the skull is a permanent volume and the brain is enclosed by rigid meninges, therefore, alterations in the volume of the intracranial components will affect ICP^[29]. The intracranial components include blood, cerebrospinal fluid (CSF), and brain tissue, all of which are relatively constant. An enlargement in one component or development of a mass lesion will elevate ICP and require a diminishing in another component in order to preserve the permanent intracranial volume^[30]. An expanding lesion can initially shift CSF and blood out of the cranium without much change in ICP. However, this capacity to compensate for changes in volume has limitation. If the lesion continues expansion, ICP will get elevated^[31]. Elevated ICP leads to cerebral herniation^[32]. Moreover, increased ICP harms CBF by depressed cerebral perfusion pressure (CPP), where CPP is calculated by subtraction of ICP from mean arterial pressure^[33].

MECHANISMS OF TTM

The multiple sites of actions are thought to be the protective effects of TTM on ischemic cascade^[34]. These multiple sites of actions include prevention of BBB disruption, reduction of oxygen derivative free radical release, reduction of excitotoxic neurotransmitter production, anti-inflammatory action and delayed apoptosis^[35]. The major neuroprotective effect of TTM in patients after cardiac arrest with restored of systemic circulation (ROSC) is apparently the protective effect on reperfusion damage^[36]. Numerous effects resulted from reperfusion damage, including oxygen free radical production, excitotoxic neurotransmitter release, and calcium influx, are all diminished by TTM^[5,6,34]. Moreover, TTM also reduces cerebral metabolic rate, protects mitochondrial break down and prevents cell membrane leakage^[37,38]. The neurons and glials are finally prevented to turn apoptosis^[38]. Protection of BBB damage is an important action of TTM^[39]. Diminution of BBB disruption helps to

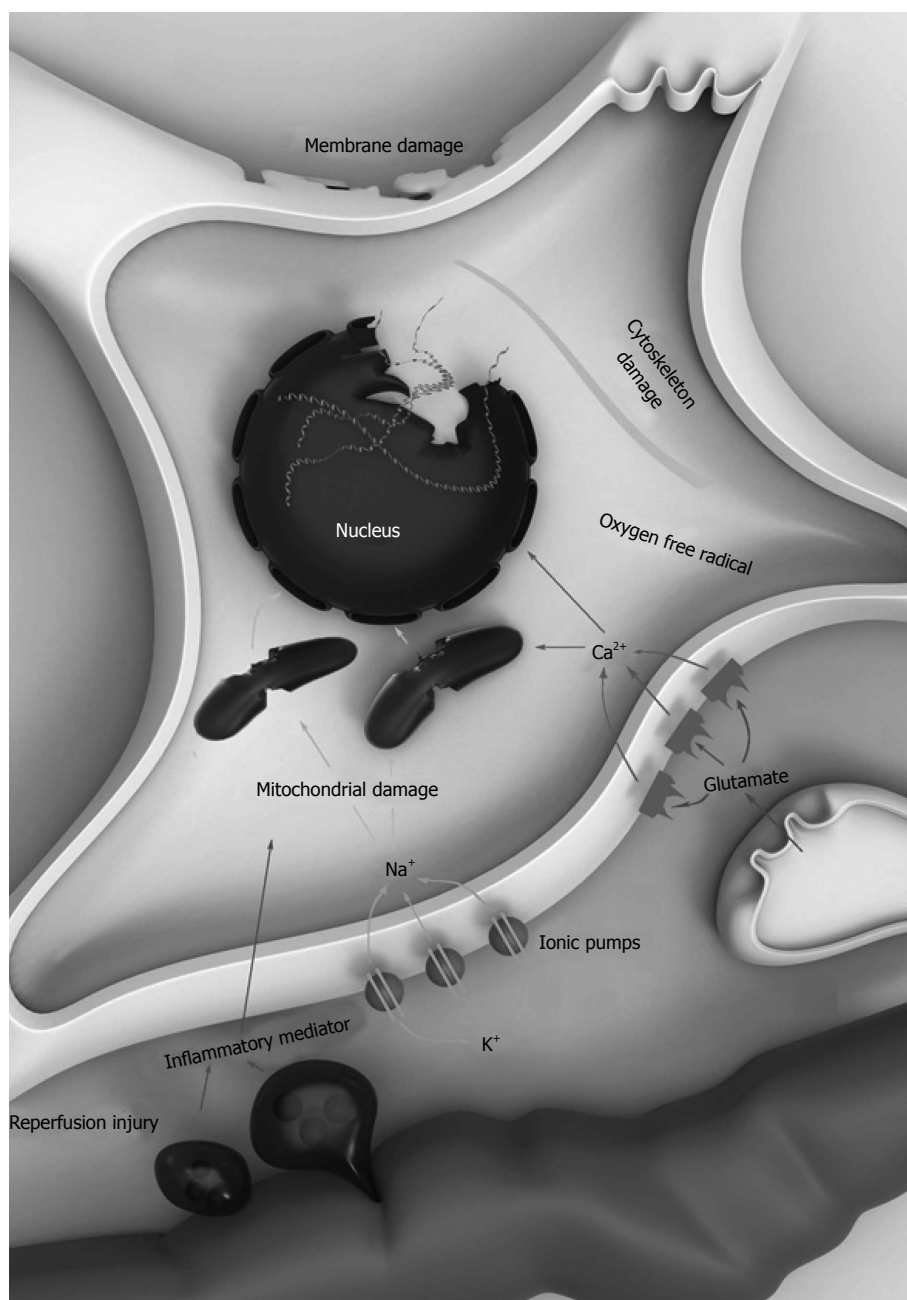


Figure 1 Hypoxic/ischemic cascade^[44] (modified from ref. [44], use with permission).

reduce brain edema then lower ICP^[38]. Effectiveness of ICP reduction by TTM in various brain disorders has been demonstrated in many clinical and experimental studies^[12,40-43]. However, absolute profit of ICP control by TTM in diverse clinical features needs to be confirmed with large scale RCTs^[3,13].

THE IDEAL TTM

The course of TTM is divided into three phases^[44]. The beginning of TTM is known as induction phase. The main idea of induction phase is to lower the current core temperature to the target as fast as possible^[45,46]. Subsequently, that target temperature is smoothly maintained for certain duration (usually for 24 h), the

so-called maintenance or sustainment phase^[45,46]. The last part, the so-called rewarming phase, the core temperature is slowly raised to the ordinary point with actively control rate, usually at 0.2-0.5 °C/h^[45,46]. Most of the important complications, particularly infection, usually happen during this last phase when the temperature is passively rewarmed with too rapid and out-of-control rate^[4]. The ideal temperature curve of a patient with cardiac arrest treated with TTM is showed in Figure 2.

METHODS TO ACHIEVE IDEAL TTM

Many methods of TTM have been reported in the literature. Some methods are no longer utilized in

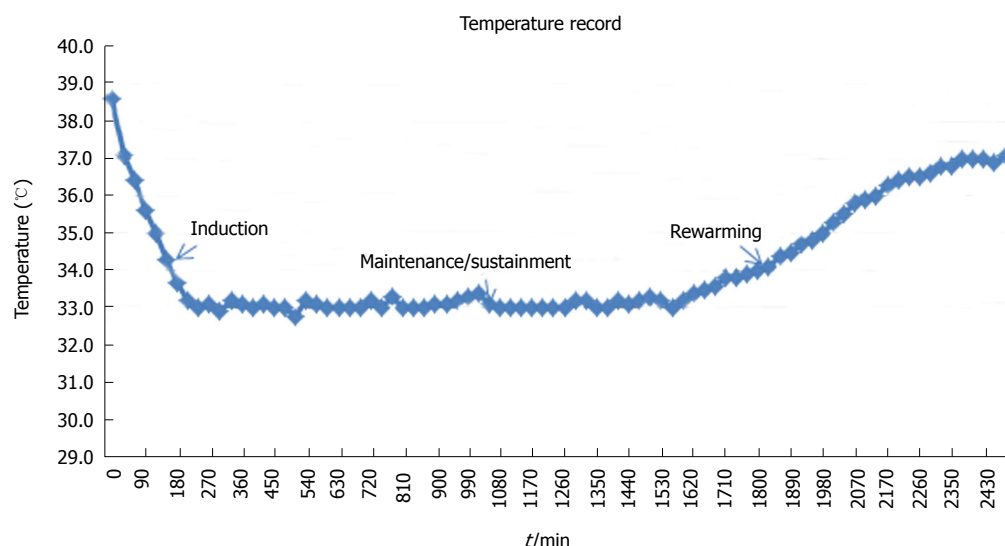


Figure 2 Temperature record of a patient with post-cardiac arrest with targeted temperature management.

clinical practice any more due to their unfeasibility or their ineffectiveness. The method with antipyretic drugs alone are, of course, not sufficient to achieve ideal TTM^[47]. Under lack of electricity source circumstance, intravenous cold crystalloid solution may be helpful for initiation of TTM during pre-hospital period^[48,49]. However, large volume is needed for induction phase. It is still not possible to achieve ideal TTM with intravenous cool fluid alone. Cooling helmets or hoods is effective to achieve selective cerebral TTM in infants however it seems to be ineffective in adults^[6,50]. The two most accepted methods in clinical practice and major clinical trials are non-invasive surface technique and invasive endovascular technique^[51,52].

Invasive endovascular methods

The hallmark of invasive endovascular techniques is central venous catheter with extracorporeal cooling machine^[4]. The central venous catheter can be stucked *via* femoral, jugular or subclavian vein. Of course, the auto-feedback temperature regulated system is integrated with the machine. Two commercial brands are obtainable in universal market: CoolGard 3000® and Celcius Control System®. The advantage of endovascular system is effective performance including rapid temperature reduction to the target, smoothly sustainment of target temperature and rewarm with actively controlled rate^[53,54]. Application of non-pharmacologic shivering control with skin counter-warming is much convenient and more effective during endovascular cooling^[55]. Without sedative effect from pharmacologic shivering control, intubation for airway protection can be avoided under skin counter-warming^[56]. That's why endovascular method is the recommend technique in several studies of TTM in subjects with acute ischemic stroke^[57-59]. However, catheter-related complications and limitation of central venous access are disadvantage

issues for endovascular method^[51,60].

Non-invasive surface methods

Compression of ice packs to neck, axilla and groin is the simplest way for surface cooling. Two landmark randomized-controlled-trials (RCT) for TTM in patients with PCAS demonstrated effectiveness of this ice packs application^[1,2]. However, disadvantage of this technique is awkward, required strenuous staff effort, unreliable temperature control and high risk for complications^[61]. The auto-feedback temperature regulated machine provides reliable temperature management and is favorable to perform in clinical practice^[47]. The machine comes with circulatory cold water blankets/pads or cold air-flow blankets. Several trademarks of machine are commercially distributed in the worldwide market, including ArcticSun®, CritiCool® and Blanketrol®. The effective automatic cooling system with temperature feedback of the machine helps rapidly lower the temperature to the target and supports slowly rewarm back to the normal baseline temperature. Core temperature monitoring straight connected to the machine is the key for auto-feedback temperature regulated system. The temperature of water within the blankets or pads is automatically regulated by the machine upon target temperature setting and feedback data from core temperature measurement^[52]. The surface method with cold water pads is showed in Figure 3.

EMCOOL® pads consist of graphite elements, the high heat conductivity, for cooling media which apply right to the superficial skin. This pads have to get frozen up in ordinary freezer to become 9 °C before application however do not require power supply while using^[62]. Consequently, this system is extremely practical in pre-hospital situation for TTM induction^[63].

The novel esophageal cooling device, the most recent non-invasive method, shows preliminary benefit



Figure 3 A patient is undergoing targeted temperature management with cold water pads.

of its use in PCAS patients^[64]. The United States Food and Drug Administration has already approved this device^[65].

SHIVERING AND COMMON PHYSIOLOGIC RESPONSE

Peripheral vasoconstriction is the initial physiologic response when temperature begins to go down^[66]. When temperature declines to the certain point, shivering usually occurs^[4]. Occurrence of shivering may represent intact physiologic response and indicate good neurologic outcomes^[67]. Wonderful shivering control is a key of success to achieve ideal TTM and should be included in the treatment protocol^[55,68]. Shivering is usually monitored with the Bedside Shivering Assessment Score during TTM (Table 1)^[69]. Elevated peripheral vascular resistance during induction phase of TTM is usually transient and takes no effect to systemic blood pressure^[70]. Sinus bradycardia with heart rate less than 50 beats per minute occurs in almost 50% of patients with PCAS during maintenance phase^[71]. Nevertheless, this bradycardia should also indicate an intact physiologic response, does not require any treatment due to no hemodynamic effect and may predict good prognosis^[72]. Platelets dysfunction and coagulation defect are hematologic abnormalities associated with hypothermia found in non-human experimental models^[4,66]. However, abnormal bleeding associated with TTM is infrequently found in real world clinical practice^[3,73]. Hypothermia also obliges kidneys to excrete water leading to volume reduction^[74]. Serum potassium becomes lower during maintenance phase due to intracellular shift and renal loss however it is expected to be elevated once temperature goes up in rewarming period^[4]. Serum amylase becomes elevated when temperature declines, nonetheless, this high serum amylase does not cause pathologic pancreatitis at all^[75]. Although elevated blood sugar due to lower insulin level usually occurs during maintenance phase, supplementary insulin may worsen the pre-existing

hypokalemia^[76]. Infection, particularly pneumonia and sepsis, is a well-known adverse event in patients treated with TTM, however it is usually not associated with unfavorable outcomes^[47,77].

APPLICATION OF TTM IN VARIOUS CLINICAL ENTITIES

TTM in PCAS

Combination of complex pathophysiologic process after resuscitated from cardiac arrest, known as PCAS, attribute to multiple organs damage^[78]. Global ischemic cascade occurs in the brain due to generalized and severe ischemia during cardiac arrest along with reperfusion process after return of spontaneous circulation (ROSC) leading to hypoxic/ischemic brain injury^[79-81]. This global brain damage is responsible for a major cause of mortality in patients with PCAS pertaining to 68% of out-of hospital cardiac arrest (OHCA) and 23% of in-hospital cardiac arrest (IHCA)^[82]. TTM is a well-known neuroprotective therapy for ischemic/hypoxic brain injury^[83-85].

Two landmark (RCT) show that induced mild hypothermia can reduce mortality rate and improve neurologic outcome in adult patients who remained comatose after resuscitated from out-of hospital cardiac arrest and had ventricular fibrillation (VF) or ventricular tachycardia (VT) as initial cardiac rhythm^[1,2]. The benefit from these two RCTs is excellent with number-needed-to-treat (NNT) 7 for avoidance of mortality and NNT 6 for favorable neurological/clinical outcomes^[86]. The summary of the two landmark RCTs is revealed in Table 2. Base on the results from these two RCTs, International Liaison Committee on Resuscitation and American Heart Association declared, in 2003 and 2010 respectively, that unconscious adults who become ROSC following OHCA with VT/VF or shockable rhythm should be treated with TTM under target temperature between 32 °C and 34 °C for 12 to 24 h^[6,7].

The appropriate target temperature for TTM in

Table 1 Bedside Shivering Assessment Score^[69]

0	No shivering
1	Mild: Shivering confines to cervical and/or thorax only
2	Moderate: Shivering extends to whole movement of upper limbs
3	Severe: Shivering spreads to overall movement of trunk, upper limbs and lower limbs

adult patients with PCAS then becomes an important dilemma. TTM Trial is a landmark RCT for comparing benefit of TTM in adult patients after OHCA with any initial rhythm at 33 °C vs 36 °C^[87]. In November 2013, TTM Trial concludes the same benefit of neurologic outcomes and survival at six months in adult patients with OHCA treated with TTM at 33 °C vs which of 36 °C^[88]. Furthermore, at six months after discharge from the hospital, survivals in 33 °C and 36 °C group have similarly good quality of life and same level of cognitive function^[89].

Clinical profit of TTM in patients with PCAS from other etiologies except OHCA with shockable rhythm remains not well-established^[90]. Some small clinical trials report evidence of marginal outcomes benefit in OHCA subgroup with asystole/pulseless electrical activity, the so-called non-shockable rhythm, and also in IHCA subgroup^[6,7,90]. For patients after OHCA with non-shockable rhythm, few observational studies show no difference in neurologic outcomes with TTM but possible reduction of mortality at six months^[2,91,92]. A recent observational study included more than 90% of adult patients with non-shockable rhythm show improvement of neurologic outcomes and better survival to hospital discharge with TTM^[73]. For patients with IHCA, few observational studies show marginal benefit of TTM in both neurologic outcomes and survival^[73,93]. The most update recommendation declared by International Liaison Committee on Resuscitation, American Heart Association and European Resuscitation Council similarly state in 2015 that unconscious adult patients with ROSC after either OHCA or IHCA with either shockable or non-shockable rhythm should be treated with TTM at 32 °C to 36 °C for at least 24 h^[8-10]. From the recent meta-analysis, TTM confers to better neurological outcomes than no temperature management in adult patients with PCAS, however, TTM in specific subgroup including initial non-shockable rhythm, IHCA and non-cardiac causes of arrest does not have sufficient data to make any conclusion^[94]. The inclusion and exclusion criteria for TTM in adult patients with PCAS at Thammasat University Hospital are showed in Table 3.

Therapeutic Hypothermia after Paediatric Cardiac Arrest (THAPCA) Trial is a landmark study for TTM in all aspects of pediatric patients with PCAS^[95]. The results from THAPCA trial demonstrated that TTM with target temperature of 33 °C in pediatric patients with PCAS due to either OHCA or IHCA did not show any outcomes benefit as compared with which of target temperature of 36.8 °C^[96,97].

Table 2 Summary of the landmark randomized control trials for targeted temperature management in post-cardiac arrest syndrome

	Australian trial	European trial
Sample size	<i>n</i> = 77	<i>n</i> = 275
TTM vs untreated	43 TTM vs 34 untreated	137 TTM vs 138 untreated
Initial rhythm	VT/VF	VT/VF
Method of TTM	Surface with ice packs	Surface with cooling blankets/pads and ice packs
Place of initiation	Emergency department	Prehospital setting
Target temperature	33 °C	32 °C-34 °C
Duration of TTM	12 h	24 h
Time of Follow up	30 d	6 mo
Outcomes	NNT of 7 to avoid death	NNT of 6 to improve neurological outcomes

TTM: Targeted temperature management; VF: Ventricular fibrillation; VT: Ventricular tachycardia; NNT: Number-needed-to-treat.

TTM in ischemic stroke

In non-human experimental models with on focal brain ischemia, TTM demonstrates a very capable neuroprotective outcomes^[98]. However, application of TTM in patients with ischemic stroke still has a lot of limitations^[99]. Invasive endovascular method is preferred to apply in patients with acute ischemic stroke due to its feasibility and safety as reported by most clinical studies^[56,100]. Under endovascular method, shivering control is convenient with non-pharmacologic skin counter-warming technique^[55]. For this reason, pharmacologic anti-shivering technique which usually associated with sedative effect can be avoided^[54,100]. Endovascular method is apparently not associated with bleeding complications even in post-thrombolytic condition^[100]. Unfortunately, the RCT of TTM with endovascular method at 33 °C following intravenous recombinant plasminogen activator (rtPA) in patients with ischemic stroke (ICTus 2 Trial) is early stopped due to the approval of interventional thrombectomy and lack of funding^[101]. The sample size of ICTus 2 Trial is too small to make any conclusion on efficacy or clinical outcomes of the treatment^[101].

With its reperfusion protective action, TTM should be useful to decrease symptomatic intracerebral hemorrhage (sICH) after intravenous rtPA as well as after endovascular treatment^[102,103]. The landmark RCT of TTM as neuroprotective treatment with target temperature at 34 °C to 35 °C in patients with acute ischemic stroke (EuroHYP-1) is still ongoing^[104]. At this moment, routine application of TTM in patients with acute ischemic stroke is not recommended^[105].

Fever control with TTM technique, to keep target temperature less than 37.5 °C, is helpful for patients with acute ischemic stroke^[106]. Reduction of ICP with TTM in malignant brain infarct is demonstrated in both experimental and clinical studies^[40,42,107]. TTM is helpful

Table 3 Inclusion and exclusion criteria for targeted temperature management after cardiac arrest at Thammasat University Hospital

Inclusion criteria
Witnessed arrest
Any initial rhythm, However initial rhythm VF or pulseless VT is the first priority
Time to ACLS was less than 15 min and total of ACLS time less than 60 min
GCS of 8 or below
SBP of > 90 with or without vasopressors
Less than 8 h have elapsed since ROSC
Exclusion criteria
Pregnancy
Known functional dependence
Down time of > 30 min
ACLS preformed for > 60 min
Known terminal illness
Comatose state prior to cardiac arrest
Prolonged hypotension (<i>i.e.</i> , MAP < 60 for > 30 min)
Evidence of hypoxemia for > 15 min following ROSC
Known coagulopathy that cannot be reversed

VF: Ventricular fibrillation; VT: Ventricular tachycardia; ROSC: Restored of systemic circulation.

for ICP reduction in patients with large middle cerebral artery (MCA) infarct^[40]. However, routine application of TTM as ICP reduction in any type of malignant brain infarct is controversial due to insufficient support clinical data of its benefit^[3,106].

TTM in TBI

Pertaining to experimental animal models for TBI, TTM provides excellent mechanism of action in both Neuroprotection and ICP reduction^[108-110]. Two clinical trials in patients with severe TBI from China demonstrated good effect of TTM on ICP control with favorable outcomes after six months to one year^[111,112]. Unfortunately, the following meta-analysis, which includes small to medium scale RCTs before 2003, did not demonstrate any benefit to apply TTM as neuroprotective therapy in patients with TBI^[113-115]. Finally, two landmark RCTs of TTM as neuroprotective therapy in either adults or children with TBI fail to demonstrate any beneficial outcomes^[70,116,117]. Elevated ICP in patients with TBI is common and associated with poor outcomes^[118,119]. The previous TTM trials begin rewarming when the peak of elevated ICP occurs at around 48 h after onset of TBI leading to clinical deterioration^[120]. This rebound elevated ICP found during rewarming phase is assumed to be one of the key reasons of failure in previous landmark RCT^[121]. Specific group of elevated ICP in patients with TBI may get clinical profit from ICP reduction with TTM^[12]. Clinical trial of TTM according to high ICP in patients with TBI was proposed^[122]. Unfortunately, large scale RCT of TTM in specific TBI patients with high ICP more than 20 mmHg (Eurotherm3235 Trial) does not demonstrate any clinical benefit^[123]. Recent meta-analysis of TTM vs normothermia in adult patients with

TBI does not demonstrate any clinical benefit of TTM but reveal risk of developing pneumonia and cardiovascular complications associated with TTM^[124]. Large scale RCT of TTM in particular aspects of patients with TBI is still ongoing^[125]. At this moment, ordinary application of TTM in patients with TBI without clinical study is not recommended^[126].

Fever controls in neurological ICU with TTM machine

Fever is commonly found in patients admitted in Neurological ICU, increases risk of complications, and is usually associated with unfavorable clinical outcomes^[127,128]. For example, in patients with ischemic stroke, chance to develop poor outcomes increases 2.2 times in each one degree exceeding 37 °C when compared with patients who have normal temperature^[129]. Most common cause of fever in Neurological ICU is infection^[130]. Similar method of TTM can be applied for fever control in Neurological ICU^[131,132]. The commonly use techniques such as surface and endovascular are convenient and save to employ for fever control^[131,132]. Fever control in patients with septic shock with external TTM machine reduces early mortality^[133]. However, overall benefit of antipyretic therapy with external TTM in patients with sepsis is still not approved^[134,135]. Fever control in patients with acute ischemic stroke is recommended per standard guidelines^[105].

TTM in other clinical entities

TTM can apply as organ protective therapy from ischemic effect during cardiovascular surgery with circulatory arrest^[136,137]. The landmark RCT of TTM for the period of operation in patients with benign grade SAH from ruptured intracranial aneurysm (World Federation of Neurological Surgeons scale between one and three) did not show any clinical benefit with more frequent associated infection^[138]. TTM can reduce perilesional edema with favorable outcomes in animal models with intracerebral hemorrhage (ICH)^[139]. The TTM after intracerebral hemorrhage (TTM-ICH) trial is ongoing^[140]. At this moment, routine use of TTM in patients with ICH is not recommended^[141]. The prospective protocol-selected trial demonstrated potential clinical benefit of local TTM in patients with neurologically complete spinal cord injury^[142]. Experts recommend that TTM can be the option for ICP control in patients with fulminant hepatic encephalopathy particularly while waiting for liver transplantation^[143].

Application of TTM in donors demonstrates organ protective effect on kidney in recipients^[144]. This RCT is the first ever for clinical trial which demonstrates organ defensive action of TTM from hypoxic/ischemic cascade outside the brain. The process of TTM at 34 °C-35 °C in kidney donors in this study is convenient and the cost of treatment is economic^[145]. TTM in kidney donors can be its second class I recommendation per standard guidelines following post-cardiac arrest in the near future.

CONCLUSION

Two main purposes of TTM in patients admitted in Neurological ICU are neuroprotective therapy and ICP control. TTM is the most potent neuroprotective treatment due to its numerous effects against ischemic/hypoxic injury. TTM provides reliable ICP reductive action. Two most popular methods of TTM using in clinical practice and clinical trials are invasive endovascular technique and non-invasive surface cooling. Fast induction, smooth maintenance and slow rewarming are the important steps to achieve ideal TTM. The strongest clinical benefit of TTM is the excellent outcomes with neuroprotective effect in patients with PCAS. TTM has been recommended as the essential treatment for OHCA with shockable rhythm for more than 10 years. Even with marginal benefit, TTM is still recommended for non-shockable rhythm and IHCA subgroup. TTM may give benefit in patients with acute ischemic stroke however its role needs to be proved with large scale RCT. TTM should be clinically useful for ICP reduction in patients with malignant MCA infarct. Routine use of TTM in patients with TBI as neuroprotective therapy or ICP control is still not recommended due to lacking of any benefit from many RCTs. TTM machine can be applied as fever control in patients with various conditions in Neurological ICU. Fever control should help to improve clinical outcomes in patients admitted in Neurological ICU.

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Basic Study

Nutech functional score: A novel scoring system to assess spinal cord injury patients

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Abstract

AIM

To develop a new scoring system, nutech functional scores (NFS) for assessing the patients with spinal cord injury (SCI).

METHODS

The conventional scale, American Spinal Injury Association's (ASIA) impairment scale is a measure which precisely describes the severity of the SCI. However, it has various limitations which lead to incomplete assessment of SCI patients. We have developed a 63 point scoring system, *i.e.*, NFS for patients suffering with SCI. A list of symptoms either common or rare that were found to be associated with SCI was recorded for each patient. On the basis of these lists, we have developed NFS.

RESULTS

These lists served as a base to prepare NFS, a 63 point positional (each symptom is sub-graded and get points based on position) and directional (moves in direction BAD → GOOD) scoring system. For non-progressive diseases, 1, 2, 3, 4, 5 denote worst, bad, moderate, good and best (normal), respectively. NFS for SCI has been divided into different groups based on the affected part of the body being assessed, *i.e.*, motor assessment (shoulders, elbow, wrist, fingers-grasp, fingers-release, hip, knee, ankle and toe), sensory assessment, autonomic assessment, bed sore assessment and general assessment. As probability based studies required a range of (-1, 1) or at least the range of (0, 1) to be useful for real world analysis, the grades were converted to respective numeric values.

CONCLUSION

NFS can be considered as a unique tool to assess the

improvement in patients with SCI as it overcomes the limitations of ASIA impairment scale.

Key words: Spinal cord injury; American Spinal Injury Association's Impairment Scale; Nutech functional score; Comparison of assessment; Positional scoring system

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Core tip: Spinal cord injury (SCI) is a devastating disease which impacts the patient physically, psychologically and financially. American Spinal Injury Association's (ASIA) Impairment Scale is a universally accepted scale to assess the SCI, but this scale does not cover all parameters of SCI. The present study focuses on the development of a new scoring system called nutech functional score for patients with SCI and compare it with internationally used scoring system ASIA.

Shroff G, Barthakur JK. Nutech functional score: A novel scoring system to assess spinal cord injury patients. *World J Methodol* 2017; 7(2): 68-72 Available from: URL: <http://www.wjgnet.com/2222-0682/full/v7/i2/68.htm> DOI: <http://dx.doi.org/10.5662/wjm.v7.i2.68>

INTRODUCTION

Spinal cord injury (SCI) is a neurological injury that affects conduction of sensory and motor signals across the site(s) of lesion(s) and the autonomous nervous system leading to long-lasting degeneration of locomotor and sensory neurons below the point of injury^[1]. Spinal cord is generally injured during work or recreation related mishaps, motor vehicle accidents as well as violence^[2]. According to factsheet of the World Health organization (November 2013), around 250000 and 500000 persons are estimated to suffer from SCI each year^[3]. Though survival over SCI has improved, yet it is of paramount importance to focus on the issue of assessment of SCI patients as perfectly as possible^[2].

There are many international standards available for examination of the neurological damage. "American Spinal Injury Association's (ASIA) Impairment Scale" is a universally accepted scale which consolidates "scores" and assesses the extent of injury, and the overall condition in its own way. These conventional scoring scales examine sensory and motor levels on right and left sides, sensory scores using pin prick and light touch, motor scores for upper and lower limb, etc. They precisely determine neurological levels, the extent of incomplete injury and achieve more consistent and reliable data^[1,4-6].

The ASIA impairment scale is a categorical scale which classifies the extent of SCI injury as motor complete and motor incomplete using grades A, B, C, D and E. "A" refers to complete injury where no function,

neither sensory nor motor, has been preserved in the sacral segments S4-S5. "B" is assigned to SCI patients where no motor function is preserved below the neurological level and sacral segments S4-S5, whereas, sensory function is preserved. The SCI patients who are diagnosed with motor incomplete condition, i.e., motor function is preserved below the neurological level, and more than half of key muscle functions below the single neurological level of injury have a muscle grade < 3, are assigned with grade "C". "D" refers to the motor incomplete condition where motor function is preserved below the neurological level, and at least half or more of key muscle functions below the neurological level have a muscle grade > 3. "E" refers to the normal condition of the patient, where sensory as well as motor functions are normal^[1]. There is a direction of BAD → GOOD from A to E, where → stands for "to" or "moves towards". The routes are distinctly two and they are A → B → E and A → C → D → E^[7]. These routes only allow counting of ASIA impairment scale which are neither numeric nor ordinal. They disconnect the ability of the sensory symptoms from the motility of the motor symptoms, as both run along two different routes. This confines the analysis with clinical research to count and rank in two streams of the scores. It was refined/improved on various basis from 1989 to 2013 which led to addition of some more parameters, such as T3 sensory examination, motor examination, testing position, wrist extension, hip flexors, ankle dorsiflexors, long toe extension, anorectal examination, etc.^[4,5,8]. However, there are many parameters such as bed sore assessment, improvement assessment, breathing pattern, etc. that are important to assess in case of SCI patients but are not covered under ASIA impairment scale yet.

The present study focuses on the development of a new scoring system called nutech functional scores (NFS) for patients with SCI and compare it with the internationally used scoring system ASIA. All the important parameters that are missed out in ASIA scale have been included in NFS that makes it more valuable in assessing the complications and improvement after treatment in patients with SCI.

MATERIALS AND METHODS

The symptoms, either common or rare, with which the patients were evaluated, were recorded in the diagnostic history. We started preparing a list of all the possible symptoms from the diagnostic history of the patients. These lists are revised time and again to maintain accuracy and precision and are used to assess patients with SCI.

Each symptom had five ordinal grades in BAD → GOOD direction. We assessed the SCI patients simultaneously with ASIA impairment scale and our new scoring system. The study was approved by Institutional Review Board of Nutech Mediworld.

Table 1 Conversion table from categorical grades to numeric range for nutech functional score

No. of scores	Numeric (Y _n)	Categorical scores (Y _c)				
		1	2	3	4	5
5	Score (Y _n)	0.122	0.310	0.500	0.690	0.890
	Range (Y _n)	0-0.241	0.241-0.379	0.379-0.621	0.621-0.759	0.759-1.00
3	Score (Y _n)	0.167	0.5	0.833	-	-
	Range (Y _n)	0-0.333	0.333-0.667	0.667-1.00	-	-

RESULTS

We developed a 63 point grading system which consisted of five grades in number for each parameter. For non-progressive diseases, 1, 2, 3, 4, 5 denote worst, bad, moderate, good and best (normal), respectively. The symptoms that are found not to be associated with SCI are scored as not afflicted in SCI (NA). Supplementary table presents the parameters assessed with NFS along with their grades. NFS for SCI has been divided into different groups based on the affected part of the body being assessed, *i.e.*, motor assessment (shoulders, elbow, wrist, fingers-grasp, fingers-release, hip, knee, ankle and toe), sensory assessment, autonomic assessment, bed sore assessment and general assessment.

The hypothetical spread of five symptoms ranging in (0.5, 5.5) were treated as equidistant to each other and were continuous. As probability based studies required a range of (-1, 1) or at least the range of (0, 1) to be useful for real world analysis, the grades were converted to respective numeric values. The "0.5" and "5.5" in the range of (0.5, 5.5) was treated as "0" and "1" of the (0, 1) in numeric scale, respectively. The configuration used to convert the range (0.5, 5.5) to the range (0, 1) demonstrated the internal consistency of the two methods of grading. It is now universal and usable for one symptom. An equation has been derived using the polynomial smoothing and graphical methods for converting categorical scores into numeric scores. The equation is as follows:

$$Y_n = 0.096 \times (Y_c + 0.5) - 0.166$$

where, Y_n = numeric score and Y_c = categorical score.

Table 1 explains the layout of the conversions. Depending upon the symptoms of parameters assessed by NFS, the five/three categorical grades in the range (0.5-5.5) can be converted to five/three numeric grades in the range (0, 1), respectively.

DISCUSSION

The spinal cord is the major conduit through which motor and sensory information travels between the

brain and body. It can get injured which leads to SCI affecting the smooth functioning of the body^[1]. Though, last decade reveals various reports emphasizing the medical management of SCI, still, there is no effective treatment to completely cure SCI. The pathophysiology, either primary injury phase or secondary, involved in SCI is essential to determine the type of possible therapeutic application that can be used after SCI.

Preceding clinical management, it is essential to determine the extent of injury. There are various scales to determine the cord segments affected by SCI^[1]. ASIA impairment scale is such a tool where its grades relate directly to a case and form categorical distributions of frequencies.

Though, many revisions have taken place in ASIA impairment scale scoring system, few limitations have been observed during assessment of SCI which restrict/limit its use. It doesn't specify the classification score for SCI patients who have patchy motor and sensory functions intact, irrespective of the level. It does not specify if motor or sensory function is non-contiguous or on one side of the body. It gives the classification of function affected below the level of injury, but doesn't describe the gross condition of the patients, such as if breathing is affected; if the patient can sit without support or even maintain the sitting posture. A study by Gündoğdu *et al*^[9] reported that the ASIA impairment scale to assess the recovery in SCI patient, it can lead to incorrect diagnosis as it may show the worsening of the condition despite of the neurological improvement of the patient. Thus, we may retrieve at an incorrect conclusion when AIS grade is considered alone without observing any motor or sensory changes during recovery^[9]. Determination of motor levels and differentiation between AIS B and AIS C/D is one of the most difficult classification tasks in AIS scoring system^[10].

The major addition made in NFS is the improvement assessment parameter. It documents even the slightest improvement by using parameters which redefined the motor and sensory functions, thereby overcoming one of the important existing limitations of ASIA impairment scale.

In our previous study, we reported several signs of improvement in our study patients who did not show any improvement when assessed with ASIA scale. Their score remained "A", both before and after the therapy. But, these patients showed improvement in sensation of fullness of bowel and bladder and control over bowel and bladder^[11]. Thus, ASIA scale lacks in assessing these parameters. Other parameters such as bed sore number and size, breathing and swallowing pattern, deformity, sweating below the level of injury, spasticity, deformity, sitting balance, standing balance, flaccidity, bulk/limb atrophy, walking distance and other general assessments including requirement of gait with calipers, calipers for standing and mobility aid, *etc.* are also not assessed by ASIA, but are included in our

Table 2 A hypothetical example showing nutech functional scores of a spinal cord injury patient before and after therapy

Parameters	NFS score before therapy	NFS score after therapy
Motor assessment (shoulder)		
Flexion	1	4
Extension	1	3
Adduction	2	5
Abduction	NA	NA
Internal rotation	2	5
External rotation	1	5
Motor assessment (elbow)		
Flexion	NA	NA
Extension	NA	NA
Supination	2	5
Pronation	2	5
Motor assessment (wrist)		
Flexion	1	3
Extension	1	4
Radial deviation	2	5
Ulnar deviation	NA	NA
Motor assessment (fingers - grasp)		
Use full palmar grasp	NA	NA
Use radial-digital grasp	NA	NA
Use standard pincer grasp	2	5
Use spherical grasp	3	5
Use intrinsic-plus grasp	3	5
Use power grasp on tool	2	5
Motor assessment (fingers - release)		
Release object freely	1	5
Release 1-inch object in container	1	5
Stack 1-inch blocks	1	5
Release tiny objects	1	3
Throw small ball at least 3 feet	1	4
Motor assessment (hip)		
Flexion	NA	NA
Extension	NA	NA
Adduction	1	3
Abduction	1	5
Internal rotation	1	5
External rotation	2	5
Motor assessment (knee)		
Flexion	1	3
Extension	1	3
Motor assessment (ankle)		
Plantar flexion	NA	NA
Dorsi flexion	NA	NA
Inversion	NA	NA
Eversion	NA	NA
Motor assessment (toe)		
Flexion	1	4
Extension	1	5
Sensory assessment		
Superficial sensation	2	5
Deep sensation	2	5
Autonomic assessment		
Bladder sensation	NA	NA
Bladder control	1	4
Bowel sensation	1	5
Bowel control	2	5
Blood pressure assessment	3	5
Bed sore assessment		
Bed sore number	1	5
Bed sore size	1	5
General assessment		
Breathing	NA	NA
Sweating	NA	NA
Swallowing	2	5
Gait with calipers	NA	NA

Calipers	1	5
Spasticity	1	5
Clonus	NE	NE
Deformity	1	5
Contracture	NA	NA
Flaccidity	3	5
Bulk/limb atrophy	1	4
Sitting balance	2	5
Standing balance	NA	NA
Walking aid	1	5
Walking distance	NA	NA
Total	67	197

NFS: Nutech functional scores; NA: Not afflicting; NE: Not existing.

newly developed NFS scoring system. This has led to a complete assessment of the patient's condition which is lacking in ASIA impairment scale.

All the parameters in NFS scoring system are graded on a scale of 1 to 5 in the range of 0.5 to 5.5, *i.e.*, NFS is ordinal, which provides complete information regarding the condition of the patients before and after the therapy. It is important to note that NFS does not include "0" as a grade. Analytical work based on "count" stays unaffected.

Let's take a hypothetical example to explain how the affected parameters are graded with NFS and ASIA. Table 2 presents a detailed manner of grading a SCI patient with NFS. Addition of scores for each parameter gives us the total score. The total NFS score of the patient before therapy is 67 and increases to 197 after the therapy. This shows a remarkable improvement in the patient after undergoing therapy. When assessed with ASIA, the grade of the patient before therapy is "A" (complete) and moves to "B" (Sensory incomplete) after the therapy. ASIA score "B" is defined as "sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5, AND no motor function is preserved more than three levels below the motor level on either side of the body". Thus, it means that patients had no improvement in motor function following the therapy which is contrary to the assessment with NFS. With NFS, we have observed improvement in motor functions of shoulder, elbow, wrist, finger-grasp, finger-release, wrist, ankle, hip and toe (Table 2). In NFS, scores can be added or subtracted; therefore even slightest improvement/deterioration in the patient's condition can be assessed.

At our facility, we have evaluated the effectiveness of NFS in assessing the patients treated with human embryonic stem cell (hESC) therapy. Thus, NFS can be considered as a unique tool to assess the improvement in patients with SCI receiving the hESC therapy. However, the universal use of the NFS will help in determining its usability in assessing the improvement in patients being treated with other therapies.

In conclusion, the NFS scoring system for SCI in numeric form is an adequate instrument to examine and score the patients with SCI. The ASIA impairment scale is based on categorical descriptions which are not

comparable with a numeric based system.

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COMMENTS

Background

Spinal cord injury (SCI) is a neurological injury that affects conduction of sensory and motor signals across the site(s) of lesion(s) and the autonomous nervous system leading to long-lasting degeneration of locomotor and sensory neurons below the point of injury. There are many international standards available for examination of the neurological damage.

Research frontiers

In the current study, the authors have introduced a new scoring system called nutech functional score (NFS) for assessment of patients with SCI and compare it with the internationally used scoring system American Spinal Injury Association's (ASIA) Impairment Scale. All the important parameters that are missed out in ASIA scale has been included in NFS that makes it more valuable in assessing the complications and improvement after treatment in patients with SCI.

Innovations and breakthroughs

The authors have developed a 63 point scoring system, i.e., NFS for patients suffering with SCI. A list of symptoms either common or rare that were found to be associated with SCI was recorded for each patient. This list is the basis to develop NFS.

Applications

NFS for SCI patients is a 63 point, positional (each symptom is sub-graded and get points based on position) and directional (moves in direction BAD → GOOD) scoring system that can be used to assess patients with SCI and compare it with the internationally used scoring system ASIA. All the important parameters that are missed out in ASIA scale have been included in NFS.

Terminology

NFS is a 63 point, positional (each symptom is sub-graded and get points based on position) and directional (moves in direction BAD → GOOD) scoring system.

Peer-review

The manuscript proposes a new scoring system, for assessing the patients with SCI. It is well written.

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Establishing the presence or absence of chronic kidney disease: Uses and limitations of formulas estimating the glomerular filtration rate

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Abstract

The development of formulas estimating glomerular filtration rate (eGFR) from serum creatinine and cystatin C and accounting for certain variables affecting the production rate of these biomarkers, including ethnicity, gender and age, has led to the current scheme of

diagnosing and staging chronic kidney disease (CKD), which is based on eGFR values and albuminuria. This scheme has been applied extensively in various populations and has led to the current estimates of prevalence of CKD. In addition, this scheme is applied in clinical studies evaluating the risks of CKD and the efficacy of various interventions directed towards improving its course. Disagreements between creatinine-based and cystatin-based eGFR values and between eGFR values and measured GFR have been reported in various cohorts. These disagreements are the consequence of variations in the rate of production and in factors, other than GFR, affecting the rate of removal of creatinine and cystatin C. The disagreements create limitations for all eGFR formulas developed so far. The main limitations are low sensitivity in detecting early CKD in several subjects, *e.g.*, those with hyperfiltration, and poor prediction of the course of CKD. Research efforts in CKD are currently directed towards identification of biomarkers that are better indices of GFR than the current biomarkers and, particularly, biomarkers of early renal tissue injury.

Key words: Chronic kidney disease; Serum creatinine; Creatinine clearance; Creatinine excretion; Estimated glomerular filtration rate; Cystatin C; Renal imaging; Hyperfiltration; Biomarkers of chronic kidney disease

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Core tip: Detection of the presence and severity of chronic kidney disease (CKD) is currently based on estimates of glomerular filtration rate based on serum creatinine and cystatin C concentrations plus factors that affect the rate of production of these two biomarkers, and on albuminuria. This scheme has improved detection of CKD and monitoring its course and the effects of therapeutic interventions. However, the scheme's performance in detecting early stages of CKD and in predicting its course is poor, in general. Research in this field is directed towards finding better biomarkers of glomerular filtration rate and, particularly, biomarkers indicating early injury of the renal tissues.

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INTRODUCTION

Chronic kidney disease (CKD) has been recognized as a major health problem worldwide with rising incidence, pronounced morbidity and mortality, and rising costs^[1]. Early diagnosis, prevention and management of

CKD, including treatment of its underlying disease and prevention and treatment of medical conditions for which the presence of CKD is a risk factor, has acquired great importance for health providers^[1]. The prevalence of CKD in the United States during the years 1999-2004 was estimated to be equal to 13.1%^[2]. Reported prevalence of CKD in various regions of the world varies. For example, recent estimates using different approaches computed a CKD prevalence of 32.5% in a small subject sample in Brazil^[3], and 6.7% and 5.8% in larger subject cohorts in Romania and Poland respectively^[4,5]. The prevalence of CKD is high in populations with conditions predisposing to it. For example, CKD was detected in 38.6% of individuals with hypertension and a high prevalence of advanced age and obesity^[6].

The diagnosis of CKD is associated with important risks of disease in other organs. For example, cardiovascular disease has been recognized as a major risk associated with CKD^[4,7]. In a cross sectional study of a large number of subjects with low and middle income in 12 countries, the incidence of CKD was 14.3% overall and 36.1% in high risk individuals, while the awareness of CKD was low and the rate of detection of cardiovascular disease in patients with CKD was also low^[8]. Adverse effects of CKD on cardiac function have been reported in patients with heart failure, but preserved ejection fraction^[9], diabetics with a doubling of their serum creatinine levels^[10], and even healthy kidney transplant donors^[11]. In a study from Korea, CKD was associated primarily with increased mortality risks from cardiovascular disease, but also with risks for other morbid conditions including malignancies^[12].

Despite the universal recognition of the importance of its early detection, CKD is diagnosed late in several parts of the world^[13]. Primary care services have a major role in the diagnosis and management of CKD^[6]. Guidelines for detection and management of CKD addressed to primary care medical practitioners have been published for adult^[14] and pediatric patients^[15]. Education of the public is an important step for early management of CKD. Patients with CKD aware of its importance desire to be informed about its risks and management^[16]. Information about CKD is provided to the public in the medical press^[17]. Finally, methods for evaluation of the economic impact of CKD^[18] and for technological developments addressing the detection and prevention of early stages of CKD^[19] are studied.

In this report, we address the current methods for diagnosing CKD. The derivation, uses and limitations of these methods will be detailed. Finally, emerging methods for early diagnosis of CKD will be briefly presented.

CURRENT METHOD FOR DIAGNOSING AND STAGING CKD

Establishing the presence and degree of renal

dysfunction has been based on measuring glomerular filtration rate (GFR). The rationale for this is a rough correlation between GFR levels and clinical manifestations of renal failure. Serum creatinine level was the traditional surrogate index of GFR. Currently, the diagnosis and staging of CKD is based on estimated values of GFR (eGFR) and presence of albuminuria^[14]. The first development leading to substitution of eGFR for serum creatinine was the computation of the Cockcroft-Gault formula^[20], which estimates creatinine clearance from serum creatinine, age, body weight and gender and was used extensively in the past for the diagnosis and management of CKD. The Cockcroft-Gault formula estimates renal creatinine clearance, not GFR. The differences between these two clearances will be addressed later in this report.

The next important step in the diagnosis and staging of CKD was the development of carefully developed equations computing eGFR based on serum creatinine levels in large prospective studies in which GFR was measured by standard methods. The Modification of Diet in Renal Disease (MDRD) Study was the first one to be used for this purpose^[21]. The MDRD formulas for eGFR were subsequently reexpressed using standardized serum creatinine values^[22]. In addition to serum creatinine, the determinants of eGFR in the currently used 4-variable MDRD formula include gender, age and race (black or not black). A second 6-variable MDRD formula, which utilizes serum urea nitrogen and albumin levels in addition to the four determinants of eGFR used in the first formula, has similar performance characteristics with the 4-variable formula^[22].

A newer set of formulas, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas, was developed by combining data from several studies in which GFR was measured by standard methods^[23]. The CKD-EPI formulas, which use essentially the same determinants of eGFR as the MDRD formula, were found to be more accurate than the MDRD formula^[23]. This higher accuracy, largely, concerned the range of eGFR values greater than 60 mL/kg per 1.73 m². However, the estimates of eGFR by the two formulas do not differ substantially for patients with moderate and advanced CKD, in general. Figures 1-3 show simulated estimates of eGFR by the MDRD and CKD-EPI formulas at different serum creatinine levels and ages in various ethnic groups and genders. Figure 4 shows the comparison of eGFR values obtained from the MDRD and CKD-EPI formulas in subjects enrolled in the NHANES and MDRD studies. In patients with CKD, MDRD and CKD-EPI eGFR values are close in general.

The next step in the development of eGFR formulas was the introduction of cystatin C, which is a small molecular weight (13.3 kDa) protein produced at a steady rate from all nucleated body cells, filtered in the glomeruli and taken up and metabolized by the proximal tubules. Serum cystatin C levels were reported to be superior to serum creatinine levels as indices of GFR. The Chronic Epidemiology Collaboration

developed formulas estimating GFR from serum cystatin C levels (the CKD-EPI cystatin C equations) and from both serum cystatin and creatinine levels (the CKD-EPI creatinine-cystatin C equations)^[24]. The determinants of eGFR are gender and the level of cystatin C in the CKD-EPI cystatin C equations, and serum cystatin and creatinine, gender and ethnicity (black or other) in the CKD-EPI creatinine-cystatin C equations. eGFR formulas based on serum creatinine or serum creatinine and cystatin C were developed for specific ethnic or age groups, e.g., Chinese^[25-27], Japanese^[28,29], pediatric^[30-33] and elderly^[34] populations. Currently, several eGFR equations have been developed or are being developed^[35].

Extensive sets of guidelines base the diagnosing and staging of CKD on combinations of eGFR cut-off values and albuminuria^[36-39]. In the next section, we will discuss the applications of these guidelines. The limitations of this approach are of importance. In a population study mean measured GFR was higher in men than women but was not different between blacks and whites^[40]. The authors of this study concluded that the different incidences of renal disease between blacks and whites were not due to the baseline renal function. The differences between the various equations computing eGFR are also not due to the baseline renal factors, but are keys to understanding the limitations of these equations as will be examined later in this text.

CLINICAL APPLICATIONS OF THE VARIOUS FORMULAS ESTIMATING GFR

Formula comparisons

A number of studies compared the accuracy of various eGFR formulas in various populations and clinical conditions^[41-63]. The great majority of these studies concluded that formulas based on serum cystatin C alone or on combined cystatin-creatinine levels are superior to other formulas^[41-47,49,50,52-54,56-58,60-63]. One study found greater accuracy with the use of the average creatinine-based and creatinine/cystatin-based eGFR formulas^[51]. Another study found that in a Korean population an eGFR formula developed in a Japanese population was superior to other formulas^[55]. Two studies found superiority of different eGFR formulas in different patient groups^[42,48]. Finally, one study^[59] found that the CKD-EPI formula^[23] and a Japanese formula for eGFR based on serum creatinine^[64] are superior to measured creatinine clearance in monitoring patients receiving cisplatin in high doses.

Uses of eGFR formulas in clinical studies

The older method for the diagnosis of CKD was to compare the serum level of creatinine of a subject to a normal range of creatinine concentrations. The clear advantage of eGFR formulas over this older method is that the formulas allow earlier detection

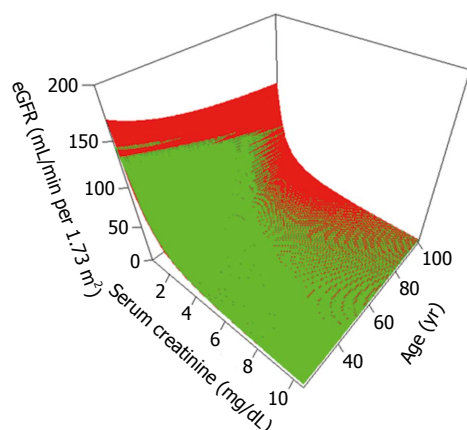


Figure 1 Modification of Diet in Renal Disease^[22] and Chronic Kidney Disease Epidemiology Collaboration^[23] formulae for estimating glomerular filtration rate fit to variations in serum creatinine (X axis) and age (Y axis) assuming males of Caucasian race. Note that the CKD-EPI formula yields slightly higher eGFR values with higher serum creatinine values and lower age whereas the MDRD formula leads to significantly higher eGFR values at very low serum creatinine values. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: Estimating glomerular filtration rate.

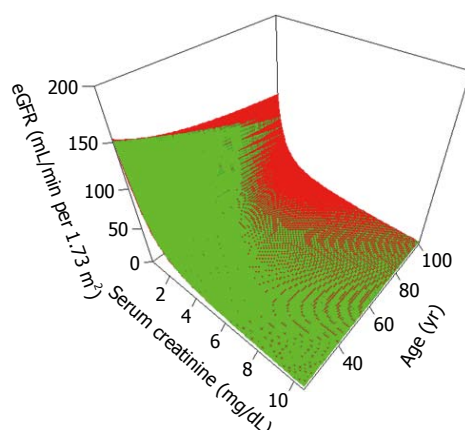


Figure 2 Modification of Diet in Renal Disease^[22] and Chronic Kidney Disease Epidemiology Collaboration^[23] formulae for estimating glomerular filtration rate fit to variations in serum creatinine (X axis) and age (Y axis) assuming females of Black race. The CKD-EPI formula yields slightly higher eGFR values with higher serum creatinine values and lower age whereas the MDRD formula leads to significantly higher eGFR values at very low serum creatinine values in this population also. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: Estimating glomerular filtration rate.

of CKD and more precise following of its course in the early stages of CKD, when large decreases in GFR lead to small rises in serum creatinine. Formulas computing eGFR have been applied in clinical studies for a variety of purposes^[43,45,46,57,58,60,65-74]. The Chronic Renal Insufficiency Cohort (CRIC) study, which is studying several aspects of CKD, is using its own eGFR formula based on serum creatinine and cystatin C levels^[65]. Estimating the incidence of CKD in populations is one area where eGFR formulas have been useful. Risk prediction in various patient groups

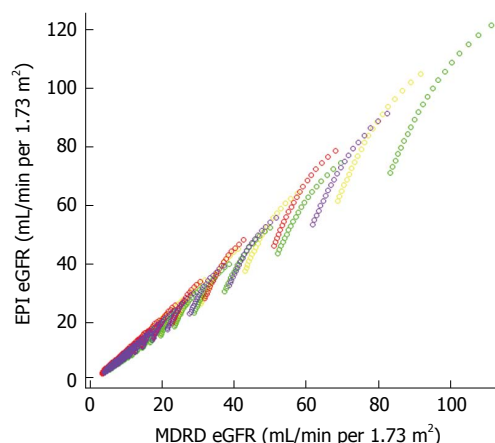


Figure 3 Scatterplot demonstrating close relationships between estimating glomerular filtration rate values calculated by the Chronic Kidney Disease Epidemiology Collaboration formula^[23] (Y axis) and the Modification of Diet in Renal Disease formula^[22] (X axis). Different colors are used to indicate the races and genders depicted in this figure: Yellow indicates Caucasian males, Green Black males, Red Caucasian females, and Purple Black females. A straight line to fit the data minimizes the least square error with an intercept of -1.03 and a beta coefficient of 1.04 achieving an R^2 value of 0.99. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; eGFR: Estimating glomerular filtration rate.

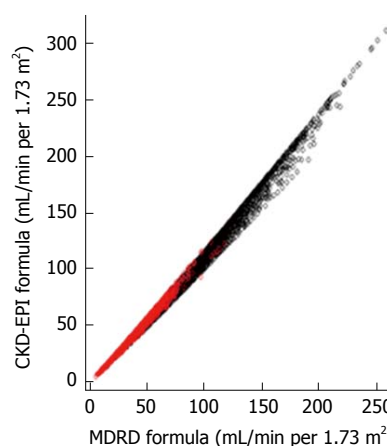


Figure 4 Comparison of estimating glomerular filtration rate values obtained by the Modification of Diet in Renal Disease^[22] and Chronic Kidney Disease Epidemiology Collaboration^[23] formulae in subjects who were enrolled in the NHANES study (Serum creatinine > 0.4 mg/dL, age ≥ 20 year) and the MDRD study^[22]. MDRD: Modification of Diet in Renal Disease; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration.

with CKD^[43,45,46,48,57,58,60,67-69,71], choice and outcome of surgical and medical interventions in patients with CKD^[56,72-74], and association of the CKD stage with specific clinical manifestations in various patient groups^[66,70] are conditions for which eGFR equations based on cystatin C or on cystatin-creatinine have been shown to provide accuracy. In clinical studies targeting specific end-points of decline in renal function, use of eGFR instead of serum creatinine has the potential of reducing substantially both the required number of participants^[75] and the targeted degree of decline in

Table 1 Clinical conditions affecting the accuracy of estimating glomerular filtration rate formulas

Diabetes mellitus
Human immunodeficiency viral infection
Chronic liver disease
Cardiovascular disease
Kidney transplants (recipients and donors)
Sarcopenia
Critical illness
Hereditary disease (e.g., Fabry's)
Obesity

renal function^[76].

LIMITATIONS OF THE FORMULAS

COMPUTING eGFR

The introduction of the MDRD formula for eGFR^[21] and the subsequent development of the current method for detecting and classifying CKD based on eGFR^[34,77,78] has enhanced CKD awareness among clinicians and the public and has created new vigor in the study of prevention and management of CKD. Nevertheless, this approach to CKD has significant limitations. This section will discuss sequentially issues with the accuracy of eGFR formulas in clinical states associated with CKD, the analysis in the literature about these issues, the main cause of inaccuracies of the eGFR formulas, and the steps required for establishing the presence or absence of CKD when an eGFR formula computes a value less than 60 mL/min per 1.73 m².

Discrepancies of the diagnosis of CKD by eGFR formulas in various clinical states

Discrepancies between various formulas estimating eGFR and between these formulas and measurements of GFR by standard methods have been reported. To illustrate the types of conditions in which eGFR formulas may be inaccurate, we will discuss a few examples of these discrepancies. Table 1 shows clinical conditions in which the accuracy of eGFR formulas has been disputed.

The increasing incidence of diabetes mellitus in many parts of the world has been the major cause of the increasing incidence of CKD. CKD secondary to diabetic nephropathy, which particularly in its early stages may not be associated with albuminuria, especially in type 2 diabetics, has its own diagnostic difficulties^[79-92]. Several studies concluded that some creatinine-based eGFR formulas are not as accurate in detecting early CKD as cystatin-based formulas^[80-84,86-88,91]. In addition, cystatin-based eGFR formulas were the only ones found to be independent predictors of diabetic complications^[83,86] and creatinine-based eGFR formulas did not detect early declines in renal function^[89,90]. One study concluded that the prediction of CKD was similar with eGFR formulas calculated by the Cockcroft-Gault formula, the MDRD

formula and a cystatin-based eGFR formula^[79]. A study using inulin clearance to measure GFR concluded that cystatin-based and creatinine-based eGFR formulas have significant inaccuracies in the diagnosis and staging of diabetic CKD^[92]. Based on the discrepancies of eGFR formulas, one report proposed the use of one of the standard techniques, iothexol clearance, for evaluation of renal function in type 1 diabetics^[85].

Infection with human immunodeficiency virus (HIV) is an important cause of CKD in several parts of the world, for example in South Africa^[93]. Proper management of patients infected with HIV requires repeated screening for CKD^[94]. Screening for CKD is of great importance for patients treated with nephrotoxic antiretroviral medications^[95]. Discrepancies between various eGFR formulas in HIV-infected patients have been reported^[96-102]. Extrarenal influences on cystatin C and creatinine metabolism may cause eGFR formula computations differing from the actual renal function^[97]. For example, serum cystatin C levels may be elevated in patients with active HIV-infection causing a large underestimation of GFR by cystatin-based eGFR formulas^[96,102]. Several eGFR formulas, based on either cystatin C or creatinine, were found to underestimate GFR in one study^[98]. Other studies in HIV-infected subjects found superiority of either cystatin-based eGFR formulas^[98,100] or creatinine-based formulas^[99] in detecting CKD and determining the risks associated with it.

Chronic liver disease is associated with inaccuracy of the eGFR formulas^[103-105]. Creatinine-based eGFR formulas systematically overestimate measured GFR in this patient group and the degree of overestimation increases with the severity of liver disease^[104]. Cystatin-based eGFR equations are more accurate in these patients^[103], but cystatin-based formulas derived in populations with liver disease may prove to have the greater usefulness^[105].

Limitations of various eGFR formulas have been reported in subjects with cardiovascular diseases^[106-109]. One study calculated similar assessment of cardiovascular risks by the Cockcroft-Gault formula and by serum cystatin C level^[106]. However, a larger study found substantial differences between eGFR values calculated by creatinine-based and cystatin-based formulas in patients with varying severity of cardiac disease, with creatinine-based eGFR values exceeding the cystatin-based values in most patient categories^[107]. Another study concluded that measured GFR, not eGFR formulas, should be used for evaluating the relationship between retinal vasculopathy and renal disease^[108]. Differences in the association of creatinine-based and cystatin-based eGFR formulas with non-traditional cardiovascular risk factors (asymmetric and symmetric dimethylarginine blood levels, insulin resistance) in subjects without diagnosed cardiovascular disease, diabetes or CKD was reported in another study^[109]. Finally, eGFR formulas were found to be inaccurate in heart transplant recipients^[110]. The authors of this last

study proposed the use of measured GFR for assessing kidney function in this patient group.

Discrepancies between eGFR formulas have been found in recipients and donors of kidney transplants. A recent study concluded that creatinine-based and creatinine/cystatin-based eGFR formulas are more accurate than cystatin-based formulas in renal transplant recipients^[111]. One study concluded that the MDRD eGFR formula was more accurate in detecting GFR values lower than 60 mL/min per 1.73 m² than the CKD-EPI creatinine/cystatin C formula after kidney donation^[112], while a second study concluded that creatinine-based eGFR formulas have low accuracy in evaluating renal function in prospective kidney donors^[113]. Of note is that the eGFR formulas used in this last study were derived in different ethnic groups. Various problems posed by creatinine-based and cystatin-based eGFR formulas in renal transplant recipients were reviewed by Santos and Martins^[114]. Based on these and other studies, United Networks for Organ Sharing (UNOS) require a measured creatinine clearance or GFR for evaluating the renal function of prospective kidney donors. Prospective renal transplant donors illustrate the limitations of eGFR formulas in subjects in whom the need of accuracy in establishing absence of CKD is critical.

Issues with the eGFR formulas were reported in patients with neurological diseases causing sarcopenia^[115,116], critically ill patients^[117], and patients with hereditary disease^[118]. Sarcopenia in subjects with neuromuscular disease is the source of systematic overestimation of GFR by creatinine-based eGFR formulas. Studies have found differences between various eGFR formulas in obese subjects^[119-122]. One large study concluded that cystatin-based eGFR formulas are deficient in detecting CKD stage 3 or 4 in obese subjects^[119]. In contrast, two smaller studies concluded that creatinine-based equations produce higher eGFR values than cystatin-based formulas and may lead to underestimation of the presence and degree of CKD^[120,122]. The finding that sarcopenia is highly prevalent in CKD patients leading to underestimation of the degree of obesity in this patient group^[121] provides an explanation for the discrepancies between cystatin-based and creatinine-based eGFR in obese subjects with CKD.

Applications of eGFR formulas in population studies

Several reports have analyzed the performance of various eGFR formulas in different populations^[123-133]. Several studies compared various eGFR formulas. A Scandinavian study found a substantially different prevalence of CKD with the use of the MDRD formula than with the use of the Cockcroft-Gault formula or of two cystatin-based equations^[123]. Similar findings were reported in a study from Uruguay, in which the lowest values of eGFR were found when using the CKD-EPI cystatin C equation, while the CKD-EPI creatinine-cystatin formula computed intermediate

eGFR values and the MDRD formula computed the highest values of eGFR^[129]. A study in Asian Indians, which also found lower overall eGFR values when using cystatin-based equations, noted that these equations resulted in widely varying eGFR values which affected the classification of CKD^[130]. A large study analyzing United States subjects with eGFR determination by the MDRD formula reported that only eGFR values lower than 45 mL/min per 1.73 m² yielded a high probability of CKD^[132].

In addition, studies in smaller numbers of subjects from various parts of the World compared eGFR formulas and GFR measurements by standard research methods^[124-128,131]. Discrepancies between eGFR computed by cystatin-based equations and measured GFR were found in a pediatric Canadian group^[124]. Differences between eGFR and measured GFR were also noted in an elderly Chinese group regardless of whether the formula used to compute eGFR was based on cystatin C or not^[125]. In a study of Japanese subjects, the creatinine-based Japanese eGFR formula, overestimated GFR in subjects who had poor renal function or were malnourished^[126]. In another study in elderly Chinese subjects, there were differences between several eGFR formulas and GFR, with some cystatin-based eGFR equations performing better than other equations^[127]. In a study at Mayo Clinic, combined cystatin- and creatinine-based eGFR correlated better with measured GFR than creatinine-based or cystatin-based eGFR values, but creatinine-based eGFR was found to have a better association with most risk factors than the other eGFR values^[128]. Another Mayo Clinic study comparing GFR with the CKD-EPI eGFR formulas based on creatinine and cystatin in recipients of organ transplants, patients with known CKD and prospective kidney donors concluded that the performance of various eGFR formulas based on creatinine or cystatin C was affected significantly by the clinical characteristics of the subjects^[131]. GFR in these studies was measured by technetium-99m-diethylene-triamine penta-acetic acid [^{99m}(m)DTPA]^[124,125,127], inulin^[126], or iohalamate^[128,131] clearance.

Formulas computing eGFR, in conjunction with other factors, have been found to be of use in assessing risks associated with CKD. The risk of progression of CKD was recently evaluated in a metaanalysis of studies in large numbers of subjects in North America and other parts of the World performed by the CKD Prognosis Cohort (CKDPC)^[133]. This metaanalysis concluded that formulas predicting the risk of progression developed in Canada and including eGFR, age, gender, and albuminuria, plus four serum biochemical values (calcium, phosphate, bicarbonate, and albumin) were accurate in predicting progression, with the proviso that calibration may be needed in certain parts of the World.

Commentaries on creatinine, cystatin C, and eGFR

The issues raised by various eGFR formulas as well as reference methods for measuring GFR have

been addressed in several reports^[134-142]. Conditions that may cause false values of creatinine-based or cystatin-based eGFR formulas were addressed in two reviews^[134,135]. One study analyzed factors leading to agreement or disagreement between measured creatinine clearance and creatinine-based formulas estimating renal function^[136]. The issues faced with the development of risk prediction formulas based on eGFR plus various other factors and with the applications of these formulas were explored in another review^[137].

One study found a systematic difference between iothalamate and iohexol, two standard markers of GFR in research studies: The average iothalamate clearance was 15% higher than the average iohexol clearance, while the average creatinine clearance exceeded the corresponding iohexol value by 42%^[138]. A related editorial discussed the potential effects of differences in the clearances of standard markers of GFR on the derivation of eGFR formulas^[139]. Two reports discussed the use of various indicators of GFR in pediatric^[140] and adult^[141] populations. Finally, one report presented a complex computer-based program for the diagnosis of CKD based on eGFR and pertinent clinical information^[142].

THE MAIN LIMITATION COMMON TO OF ALL eGFR FORMULAS

The section will start with the presentation of three subjects under the care of nephrologists in two hospitals in Albuquerque. These subjects illustrate issues created by eGFR formulas and suggest the proper way to address these issues. Creatinine-based eGFR values were computed by the MDRD^[22] and CKD-EPI^[23] formulas in all three subjects. In the third subject, cystatin-based and creatinine/cystatin-based eGFR were computed by the CKD-EPI formulas^[24]. All eGFR values in these patients are in mL/min per 1.73 m².

Illustrative cases

Case 1: A 61-year-old white man with quadriplegia for 25 years following a motor vehicle accident and on hemodialysis for two years transferred to an inpatient spinal cord injury unit in New Mexico from another state. Immediately prior to the first hemodialysis session, his serum creatinine level was 1.27 mg/dL, with eGFR values of 58 by the MDRD formula and 61 by the CKD-EPI formula. Serum creatinine levels ranged between 1.12 and 1.32 mg/dL throughout the dialysis period. The agency overseeing chronic dialysis facilities in New Mexico requested definitive proof of end-stage kidney disease (ESKD). A 48-h urine collection through the permanent indwelling urinary bladder catheter carried by the patient revealed the following values: Volume of collected urine 320 mL; total creatinine content in this urine specimen 62 mg.

Case 2: A 31-year-old white woman had one year

after donating a kidney a stable serum creatinine concentration of 1.32 mg/dL, with eGFR values of 47 by the MDRD formula and 53 by the CKD-EPI formula. Urinalysis was repeatedly clean and urine albumin was undetectable. She is dedicated to exercise. Creatinine excretion in a 24-h urine specimen was 1672 mg and serum creatinine collected at the end of the urine collection remained at 1.32 mg/dL. Her body surface area is 1.81 m². Calculated renal creatinine clearance was 84 mL/min per 1.73 m².

Case 3: A 71-year-old white man with hypertension under excellent control had on repeated testing a serum creatinine level of 1.60 mg/dL, no albuminuria, and ultrasound showing no abnormalities in the renal texture and no post-void residual urinary bladder volume. He is exercising intensely. Serum cystatin C level was 1.0 mg/L. Calculated eGFR was 43 by both the MDRD and CKD-EPI creatinine equations, 57 by the CKD-EPI creatinine-cystatin equation, and 74 by the MDRD-EPI cystatin C equation.

The main limitation of eGFR formulas

The main limitation of the eGFR formulas is significant lack of accuracy in individuals as compared to groups. This causes problems in establishing the presence or absence of early CKD. The inaccuracy is rooted in the method for developing these formulas and the nature of the biomarkers used in them. eGFR formulas are multiple regression equations estimating GFR values using the serum concentration of one or more biomarkers eliminated by glomerular filtration and of other factors that affect the production of the biomarkers. Factors entered in these formulas so far are ethnicity, gender and age. A group of subjects with the same GFR, ethnicity, age and gender will not have the same serum concentration of the biomarker. Instead, they will have a range of concentrations around the mean value for this group, which is the value computed by the eGFR formula. Conversely, the values of GFR will have a range around the mean value provided by the eGFR value. The width of the range determines the standard error of the eGFR estimate provided by the regression equation.

The errors of eGFR formulas derived by regression have been quantified by P30 statistics, *i.e.*, the probability that the measured GFR will differ from eGFR by 30% or less in various cohorts including the cohort that was used for the CKD-EPI formula^[143], a cohort of elderly subjects^[144] and a cohort of transplant recipients^[145]. GFR and eGFR values differ by more than 30% in approximately 20% of the individuals. The origin of this difference lies in the fact that eGFR formulas do not include all the factors that affect the serum concentration of a biomarker or account for changes in the quantitative effects of factors with varying intensity (*e.g.*, degree of sarcopenia). Indeed, development of an eGFR formula accounting for all the

influences on the steady state serum concentration of a biomarker and for quantitative variations of these influences would be an exceedingly difficult task.

The nature of the biomarkers used in eGFR formulas is also a source of differences between eGFR and GFR values. eGFR formulas are applicable only to steady states of biomarkers. In the steady state, the rates of production and removal (the total clearance) of a biomarker are equal and its serum concentration is the fraction production/clearance. An ideal biomarker for GFR would also be exceedingly difficult to find. Such a biomarker should be eliminated exclusively by glomerular filtration and have the same exactly production rate in all subjects with the same age, gender, ethnicity and whatever other factors may be entered in eGFR formulas in the future. In examining whether a biomarker is suitable as an indicator of GFR one should investigate both its production and routes of elimination. Creatinine and cystatin C fail to fulfill the criteria for ideal biomarkers of GFR.

The production of creatinine receives influences from a host of factors other than those entered in the eGFR formulas and its elimination is not only through glomerular filtration. Factors affecting the production of creatinine and not included in the eGFR formulas include level of exercise, diet, particularly red meat ingestion or intake of dietary supplements containing creatine^[146-148], neuromuscular diseases leading to loss of muscle mass, and disease states affecting the rate of conversion of creatine to creatinine^[149]. Not accounting in the eGFR formulas for factors affecting creatinine production can affect the accuracy of eGFR estimates in individuals as well as cohorts. For example, the reported larger degree of underestimation of GFR by the MDRD formula in healthy individuals than in CKD patients^[150] was probably the consequence of higher production rates of creatinine in the healthy individuals.

Creatinine production differences between subjects with and without diabetes mellitus, which is a major cause of CKD, are the reason for the inaccuracies of creatinine-based eGFR formulas discussed earlier. There is evidence suggesting that diabetes affects creatinine production. Serum creatinine levels tend to be low in diabetic individuals^[151], reflecting low rate of creatinine production, hyperfiltration (see below) or a combination of the two. Lean body mass, a large part of which is muscle mass, decreases with age rapidly in diabetic subjects^[152]. The loss of muscle mass in diabetic subjects is the source of discrepancies between GFR and creatinine-based eGFR formulas^[153]. Creatinine excretion is systematically lower in diabetic than non-diabetic subjects with ESKD treated by peritoneal dialysis^[154]. A formula developed in a peritoneal dialysis cohort includes diabetes among the predictors of creatinine excretion^[155]. Future developments in creatinine-based eGFR formulas should study, and most probably include, diabetes among the factors affecting serum creatinine concentration.

Factors other than GFR affect creatinine excretion. Renal creatinine excretion is not exclusively through glomerular filtration. Tubular secretion contributes a small part, around 15%, of the urinary creatinine at normal GFR values. In glomerulopathic CKD, the fraction of urinary creatinine excreted through tubular excretion increases progressively as GFR decreases and serum creatinine rises^[156]. Removal of creatinine through tubular secretion is the source of significant overestimation of GFR by creatinine-based eGFR formulas in CKD. In addition to tubular secretion, creatinine is removed from the body through extrarenal routes, mainly through the gastrointestinal tract. An indirect approach computed an average extrarenal creatinine clearance of 0.042 L/kg per 24-h in males and 0.041 L/kg per 34-h in females with advanced CKD^[157]. This approach suggests that progressively larger amounts of creatinine are removed through the extrarenal pathway as serum creatinine rises progressively in worsening CKD. This would cause progressive overestimation of GFR by creatinine-based eGFR formulas.

Sickle cell disease is one condition leading to large differences between renal creatinine clearance and GFR. Creatinine-based eGFR formulas have greatly overestimated true GFR in patients with sickle cell nephropathy. This is related to the supranormal proximal tubular function in subjects with sickle cell disease resulting in enhanced creatinine secretion and various electrolyte disturbances^[158,159]. It has been suggested that serum cystatin C and possibly cystatin-based eGFR formulas can be better indicators of renal function in such patients^[160]. Conditions that affect the extrarenal removal of creatinine need further study.

Several medications affect the production or tubular excretion of creatinine. Table 2 shows medications that affect creatinine production^[161,162] or block tubular creatinine secretion^[163-168]. In the past, several research studies measured GFR by creatinine clearance with the use of medications blocking tubular creatinine excretion^[163]. A great number of medications may induce myopathy leading to varying rates of creatinine production. Drugs induce myopathy by direct myotoxicity (e.g., alcohol, cocaine, glucocorticoids, statins, antimalarial compounds, colchicine, zidovudine), immunological mechanisms causing inflammation (e.g., D-penicillamine), or various indirect mechanisms (e.g., drug-induced coma causing muscle ischemia from compression, diuretic-induced hypokalemia, and drug-induced hyperkinetic syndromes, dystonic states, hyperthermia or neuroleptic malignant syndrome)^[169].

Acute drug-induced rhabdomyolysis is manifested by elevation in the serum concentration of muscle enzymes (e.g., creatinine phosphokinase) and leads to acute kidney injury in some instances, in addition to a rise in serum creatinine due to overproduction. In chronic drug-induced myopathy causing increased creatinine production, however, serum levels of muscle enzymes may not be elevated^[169]. In subjects

Table 2 Drugs raising serum creatinine concentration

Drug	Ref.
Drugs enhancing creatinine production	
Fenofibrate	[161]
Vitamin D receptor activators	[162]
Drugs inhibiting tubular creatinine secretion	
Cimetidine	[163]
Cobicistat	[164]
Dronedarone	[165]
Pyrimethamine	[166]
Salicylates	[167]
Trimethoprim	[168]

with advanced CKD, for example ESKD patients treated by peritoneal dialysis, increased creatinine production secondary to drugs can cause large rises in serum creatinine concentration and excretion without a change in creatinine clearance or in the serum levels of muscle enzymes^[170]. Finally, errors in serum creatinine values are caused by interference of various substances, endogenous or exogenous, with the creatinine assay. This issue was more common with the older method of measuring creatinine concentration in biological fluids by the non-specific Jaffe colorimetric method^[171]. However, even the newer specific enzymatic methods receive interference from other substances, including dopamine, ascorbate^[171], the analgesic dipyron (metamizol), N-acetylcysteine and other substances.

Like creatinine levels, serum cystatin C levels receive influences independent of GFR^[172-183]. Changes in renal and extrarenal function and in the production of cystatin C may affect its serum level. As noted cystatin C is filtered in the glomeruli and then reabsorbed and catabolized in the proximal tubules. Its urinary excretion is a small fraction of its filtered load. It has been postulated that tubulointerstitial disease damaging the integrity of the tubular barrier may lead to back leak of cystatin C into the peritubular blood capillaries and increase in serum cystatin C levels^[172]. A change in tubular handling of cystatin C was reported in children with nephrotic syndrome who exhibited significant rises in the urinary excretion of the compound at times of heavy proteinuria^[175]. Potential influences of renal tubular dysfunction on cystatin C serum levels will require further study.

Factors affecting the extrarenal clearance and production of cystatin C have not been studied adequately. Indirect methods to quantitate these influences have been proposed^[177]. Increases in the rate of production and the serum levels of cystatin C have been reported with the use of corticosteroids^[173], and in subjects with obesity^[180], large lean body mass^[178], hyperthyroidism^[178,183], elevated serum triglyceride levels^[181], and after coffee consumption^[182]. Two reports studied by multivariable statistical analysis factors affecting serum cystatin C levels independently of GFR^[174,179]. Older age, male gender, large weight and

height, current cigarette smoking and higher C-reactive protein levels were associated with higher serum cystatin C levels in a study from the Netherlands^[174]. A study pooling data from three large research studies identified younger age, male gender, diabetes mellitus, high levels of C-reactive protein, high white blood cell count and low levels of serum albumin as independent factors associated with high levels of cystatin C^[179]. Race had also a small independent effect on cystatin C in this last study. Finally, potential interferences with the measurement of cystatin C have not been adequately studied.

How to proceed when a low eGFR value suggest CKD in an individual

Serum creatinine is routinely measured for surveillance of the renal function, while cystatin C is not a routine blood test. Currently, the initial step for diagnosing and staging CKD is based on determination of serum creatinine and albuminuria. Creatinine-based eGFR values are of paramount importance in this process^[39]. The rate of creatinine production is the cause of questioning the accuracy of eGFR in the great majority of subjects. The suggested next step in the evaluation of individuals for whom there are questions about the accuracy of creatinine-based eGFR is to measure serum cystatin C and compute cystatin-based and creatinine/cystatin-based eGFR values^[39]. This approach, which was based on the finding that the effect of muscle mass on serum cystatin C levels is small^[179], may confirm the presence of CKD in subjects in whom the cystatin-based eGFR values agree with the creatinine-based values, but will create new problems if the various eGFR values disagree. The subject reported in case 3 above illustrates this problem.

A different approach for confirming or rejecting a questionable creatinine-based eGFR value is required. The hypothesis that an unusual rate of creatinine production led to an unusual serum creatinine level is addressed directly by measuring creatinine excretion rate^[184]. Cases 1 and 2 of this report illustrate this point. As noted, candidates for living kidney donation are a group of subjects who should also have their creatinine clearance determined. Differences between estimates of eGFR and estimates of creatinine clearance by the Cockcroft-Gault formula affecting significantly the dosing of potentially toxic drugs have been reported^[185]. Dosing of drugs may require measurement of creatinine clearance in selected cases. In addition to clarifying the information provided by creatinine-based eGFR, determination of creatinine excretion has prognostic significance. Low urinary creatinine excretion levels in CKD patients are associated with adverse outcomes^[186].

Establishing the presence or absence of CKD from creatinine clearance also has drawbacks. Urine collection and storage for 24 h is a demanding task. Errors in the timing of urine specimens and presence of obstructive urinary tract disease are sources of

inaccuracy of urine collection. Detailed explanation of the importance of accurate urine collection and detailed instruction about the timing of this procedure are imperative and minimize collection errors. Motivation of individuals with questionable eGFR values is important. Subjects with low creatinine-based eGFR values and suggestion of large muscle mass are usually very motivated to know whether they have CKD or not. Evaluation for urinary obstruction should be considered a necessary step of the diagnosis of CKD.

A third drawback of the measured creatinine clearance is the systematic overestimation of GFR because of tubular secretion of creatinine. This may become significant when the value of the measured creatinine clearance is above, but close to 60 mL/min per 1.73 m². In glomerulopathic subjects with normal GFR values, creatinine clearance exceeded by 16%, on the average, GFR measured by inulin clearance^[156]. We suggest that creatinine clearance values less than 72 mL/min per 1.73 m², that is values exceeding 60 mL/min per 1.73 m² by less than 20%, should call for further investigation. Cystatin C measurements may improve the accuracy of diagnosis of CKD in this last group of subjects, but will require further studying.

As noted, careful history taking about conditions predisposing to CKD is very important in establishing the diagnosis of CKD^[142]. Imaging techniques are also of help. Size and texture of the kidneys and features of obstructive disease of the urinary tract are routinely investigated by traditional ultrasonographic techniques. Addition of color Doppler and spectral Doppler to conventional ultrasonography allows detailed investigation of the renal circulation. Circulatory indices derived from these newer techniques, including the resistivity index and the strain index, as well as evaluation of renal fibrosis by elastography, can provide valuable assistance in the diagnosis of CKD^[187-189]. Measurement of GFR by nuclear scanning^[190,191] is another imaging technique assisting the early detection of CKD in vulnerable patient groups, *e.g.*, renal transplant recipients. Other imaging techniques applied in the diagnosis of renal diseases (*e.g.*, radiography, computed tomography, nuclear magnetic resonance methods) have limited value in the detection of CKD. Despite the proliferation of studies evaluating various biomarkers as indicators of specific renal histology, renal biopsy remains the gold standard of the histologic diagnosis of renal disease^[192]. The role of renal biopsy in establishing the presence of CKD, however, is questionable.

FUTURE DEVELOPMENTS

Two types of findings have generated questions about the use of either measured GFR or eGFR formulas for the diagnosis and classification of CKD. The first issue was the varying association between the serum levels of several small molecular weight substances classified as uremic toxins and eGFR values calculated

by several formulas in subjects with CKD stages 2 to 5^[193]. This finding, which was attributed to varying effects on the serum concentration of uremic solutes of factors other than GFR, including tubular handling, extrarenal removal and production^[194] provides a stimulus for searching for new biomarkers of eGFR better associated with various uremic toxins, but does not eliminate the current principle of diagnosis and staging CKD based on GFR. Based on the conclusions of the last report^[194], it is possible that other factors have significant effects on the serum concentration of uremic toxins independently or in addition to the presence and stage of CKD. Findings of elevated serum levels of "uremic" toxins in subjects without clinical features of CKD and normal levels of GFR measured by standard methods would provide support to this last postulate.

Hyperfiltration is the second category of findings creating questions about using eGFR or GFR to diagnose and stage CKD. Hyperfiltration has been extensively investigated in subjects with type 1 or type 2 diabetes mellitus in whom it is considered an important factor in the initiation and progression of diabetic nephropathy^[195]. Subsequently, hyperfiltration was reported in a variety of clinical conditions including obesity, hypertension, metabolic syndrome, smoking, sickle cell disease, thalassemia, IgA nephropathy, reflux nephropathy, kidney donors, transplanted kidneys, cirrhosis, pregnancy, lead poisoning, autosomal dominant polycystic kidney disease, primary aldosteronism, nephropathy from Puumala hantavirus, and apparently healthy subjects to whom it confers a risk of CKD and hypertension^[196]. It is of interest that intense exercise can decrease the prevalence of hyperfiltration in the general population^[197].

Establishing the presence of hyperfiltration is of great importance. A review of studies on hyperfiltration disclosed the use of a variety of methods for measuring or estimating GFR and a variety of GFR or eGFR cut-off values defining hyperfiltration^[198]. Hyperfiltration is defined as either supernormal GFR or increased filtration fraction (GFR over renal plasma flow), which is normally around 0.20. Elevated glomerular capillary hydrostatic pressure, with or without an elevation in the renal plasma flow, leads to hyperfiltration^[195]. Subjects with hyperfiltration on the setting of increased renal plasma flow, for example a subset of patients with early diabetic nephropathy, have supranormal GFR. A few studies of hyperfiltration have measured both GFR and renal plasma flow. In addition to its accuracy in establishing presence or absence of hyperfiltration, this method allows detection of hyperfiltration in subjects with established CKD and low GFR values.

Hyperfiltration creates two major problems with the diagnosis of CKD. The first problem is a documented lack of accuracy of various eGFR formulas in subjects with hyperfiltration^[199,200]. The second problem is even more serious and cannot be corrected by finding new biomarkers providing accurate eGFR formulas in

Table 3 New biomarkers for chronic kidney disease

Biomarker	Ref.
Biomarkers for GFR	
Symmetrical dimethylarginine	[212,213]
Beta-trace protein	[214,216,217]
β 2-microglobulin	[215-218]
Galectin-3	[219]
Biomarkers for injury of renal tissue	
MicroRNA	[220,221]
Soluble urokinase-type plasminogen activator receptor	[209,222,223]
Proteomics	[224,225]
Gelatinase-associated lipocalin	[226,227]

GFR: Glomerular filtration rate.

subjects with hyperfiltration. Even if such biomarkers are found in the future, subjects with hyperfiltration, early stages of CKD, absence of albuminuria and GFR in the normal range will be misclassified as not having CKD by the current scheme. Elderly subjects with apparently normal renal function, but with loss of nephrons and hypertrophy of the remaining nephrons^[201-203] are one such group. Detection of CKD in these subjects requires an approach other than measurement or estimation of GFR.

The limitations of the available tools for diagnosing and staging CKD have complicated the development of accurate models for early detection of CKD and prediction of its progression. One approach that has been investigated is the measurement of the renal functional reserve (RFR)^[204]. RFR, the temporary increase in renal blood flow and GFR after a standardized heavy protein meal, is a homeostatic mechanism prominent in carnivores. Healthy humans exhibit a less pronounced, but quite large RFR after a protein meal^[204]. Certain studies suggested that measurement of renal functional reserve may be a useful method in detecting early CKD. In one study, vasculopathic patients with normal GFR and absent RFR by GFR measurement developed within two years a significant decrease in GFR^[205]. In another study, the magnitude of RFR decreased progressively with higher stages of CKD^[206]. Doppler ultrasonography can assist in the evaluation of RFR^[207,208]. However, conflicting RFR findings have been reported in several studies of patients with CKD. Whether RFR measurement can provide a useful method for establishing the presence or absence of early CKD is not clear currently.

Another approach to the early detection of CKD is acquiring strength. Recent reports have stressed the need for new biomarkers that can enhance the accuracy of CKD detection^[137,209,210]. Table 3 shows biomarkers evaluated in CKD. Current practices utilize two categories of biomarkers for CKD, those that estimate GFR and those that indicate damage to a specific renal function (albuminuria). Similarly, the search for new biomarkers has two directions, indicators of GFR which are routinely assayed in the serum^[211-219] and indicators of specific types of kidney

injury which are assayed in the urine or serum^[220-227].

Demographic and clinical associations of creatinine and newer GFR biomarkers, including β -trace protein, β 2-microglobulin, and cystatin C, differ^[216]. In specific patient groups, newer GFR biomarkers may offer specific advantages. For example, cystatin C and β 2-microglobulin are more sensitive indicators of decreased GFR than creatinine in critically ill children^[215], while β -trace protein and β 2-microglobulin serum levels may identify additional risk factors in patients with CKD^[218]. However, the role of these newer biomarkers in establishing the diagnosis of CKD will need further investigation. For example, eGFR estimates from equations based on β -trace protein or β 2-microglobulin were found to be less accurate than the creatinine-based or creatinine/cystatin-based CKD EPI equations^[217].

In patients with ESKD, serum levels of biomarkers eliminated poorly by dialytic techniques can provide more accurate estimates of residual GFR than the estimation as the average of renal creatinine and urea clearances^[228]. Residual renal clearance may be estimated by pre-dialysis β 2-microglobulin or β -trace protein concentrations in patients receiving high flux hemodialysis or hemodiafiltration^[229,230]. A preliminary report identified four metabolites, including acetyl-threonine, pseudouridine, acetyl-alanine, and myo-inositol with higher correlation values with measured GFR than serum creatinine levels^[231]. eGFR formulas containing multiple metabolites appear to be more accurate than creatinine-based formulas in estimating GFR^[232-234]. Despite the promise that this strategy holds, it should be noted that even system-biology combined markers derived from a rational process only moderately improve performance relative to clinical and standard laboratory evaluations. Also, the cost of measuring these biomarkers vs measuring GFR by a standard method is an issue that will be raised.

Regardless of the potential advantages of eGFR estimates from new biomarkers, these estimates will have some of the limitations discussed above. The main progress in the diagnosis of CKD and the prediction of its course is expected to result from introduction of biomarkers of renal tissue damage in clinical practice^[221]. Note that biomarkers of renal tissue injury assayed in serum may also provide estimates of GFR and have the potential of providing clues about the histologic diagnosis of CKD^[235] and combination of clinical predictors and biomarkers can predict progression of CKD in specific patient groups, e.g., subjects with type 2 diabetes mellitus^[236].

CONCLUSION

The diagnosis and staging of CKD based on estimates of GFR from serum creatinine and cystatin C concentrations represents a major step in the diagnosis of CKD and in following its course, but has significant limitations. The main limitations of this methodology are its low discriminatory power in establishing the

presence or absence of early CKD in individuals and its unsatisfactory performance in predicting the course of CKD. The direction of Research in this field is currently towards identifying new biomarkers that either are superior indicators of GFR, or indicate early injury of the renal tissue. The last group of biomarkers has the potential of leading to improved early detection of CKD.

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Accurate diagnosis of prenatal cleft lip/palate by understanding the embryology

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Abstract

Cleft lip with or without cleft palate (CP) is one of the most common congenital malformations. Ultrasonographers involved in the routine 20-wk ultrasound screening could encounter these malformations. The face and palate develop in a very characteristic way. For ultrasonographers involved in screening these patients it is crucial to have a thorough understanding of the embryology of the face. This could help them to make a more accurate diagnosis and save time during the ultrasound. Subsequently, the current postnatal classification will be discussed to facilitate the communication with the CP teams.

Key words: Cleft lip; Cleft palate; Embryology face; Orofacial clefts; Ultrasound

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Core tip: Cleft lip/palate is a very common craniofacial malformation. Currently a thorough ultrasound examination during the 20-wk ultrasound is performed to exclude an oral cleft of the face. This study provides important embryological information to facilitate the ultrasonographer in making an accurate diagnosis and safe time during the ultrasound. Subsequently, the current postnatal classification will be discussed to facilitate the communication with the cleft palate teams.

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INTRODUCTION

Orofacial clefts (OFCs) are common craniofacial malformations. Cleft lip (CL) with or without cleft palate (CL/P) occur more commonly in males, while 1:1000 Caucasians, 2:1000 Asians, and 0.3:1000 Africans are affected^[1]. However, isolated cleft palate (CP) is more common in females and an equal incidence of 0.4:1000 live born is encountered in all races^[1]. Although the distribution for clefts differs per region it is estimated to be 20%-25% CL, 40%-50% CLP and 30%-35% CP. Clefts occur in a ratio of 6:3:1 unilateral left, unilateral right, and bilateral^[2]. The etiology of OFCs is complex and believed to be multifactorial, representing an interaction between genetics and environment during a critical stage of development^[3]. Recently several genes causing CL and palate have been discovered. The nature and function of these genes vary widely, illustrating high complexity within the craniofacial developmental pathways^[4-6]. The interested reader is referred to comprehensive studies that focus specifically on these genes.

In different countries routine ultrasound screening in pregnancy does not consistently include screening for facial clefts. However, the increased use of trans-abdominal ultrasound (3D) certainly leads to an increased frequency of oral clefts being diagnosed antenatally^[7]. There are few articles that focus on different ultrasound approaches to visualize the palate and lips both 2D and 3D^[8-10].

As oral clefts typically occur in facial areas where the normal embryological fusion of structures did not occur, knowledge of the embryological background could aid the ultrasonographer to understand and more accurately diagnose these clefts.

The aim of this review is to familiarize the ultrasonographer with the embryology of the face, which will subsequently aid in more accurate diagnosis of the extent of the facial cleft. For a more extensive overview the reader is referred to textbooks^[11-13]. The different classifications systems of clefts are also summarized. This might facilitate communication between the ultrasonographer and the CP team after birth.

DEVELOPMENT OF THE LIP

The basic morphology of the face is established between the 4th and 10th week after conception. Upper lip formation commences at 24 d postconception and is completed by 37 d^[11-13]. At five weeks' gestation, when the embryo is 3 mm long, the ectoderm in the vicinity of the neural plate folds on itself to form the neural tube. Special neural crest cells of ectodermal origin differentiate to form a special ectomesenchyme. The ectomesenchyme migrates over and around the head and participates in the formation of five facial prominences that surround the primitive oral cavity: The frontonasal prominence, the paired maxillary prominences and the paired mandibular prominences.

The frontonasal prominence develops in the midline over the brain. During the 5th week of embryogenesis the nasal component of the frontonasal prominence forms bilateral two ectodermal thickenings, the nasal placodes (Figure 1)^[13]. Each nasal placode invaginates to form an oval nasal pit and divides the frontonasal prominence into a medial and lateral nasal process. During the 6th week, the two medial nasal processes fuse and gives rise to the midline of the nose, medial part of the upper lip, philtrum, incisor teeth and the primary palate. The primary palate is the part of the palate that is located ventrally to the foramen incisivum, while the secondary palate is the part located dorsally to the foramen incisivum. The lateral nasal process subsequently forms the nasal alae and alar base.

During the 6th week the maxillary processes on each side of the mouth grow forward and merge with the medial nasal processes that lead to the formation of the lateral upper lip, the majority of the maxilla and the secondary palate. The mandibular prominences give rise to the mandible and lower lip. The fusion of the facial swellings occurs between the 4th-6th weeks postconception. Failure of fusion between any of the facial swellings results in facial clefts and can occur either unilaterally or bilaterally and typically happens at the junction of the lateral incisor and the first premolar teeth.

In patients with mild CL defects the cleft could be limited to a notch in the vermillion border of the lip that probably represents a failure of localized growth of the medial nasal process. In more severe defects, the cleft runs through all the lip structures and completely separates the lateral lip from the philtrum and nasal cavity. These clefts are caused by failure of fusion between the medial nasal process and the maxillary prominence. The depth of the cleft may vary from the soft tissue of the lip to a complete cleft of the maxillary bone. The normal palate fusion process starts at the foramen incisivum and subsequently closes in a posterior direction. The actual lip fusion starts cranially and subsequently closes in a caudal direction.

Oblique OFCs can also involve the side of the face and even involve the orbit. These clefts comprise less than 1% of facial clefts and can be classified according to Tessier's anatomical classification^[14]. Midline clefting syndromes can be divided into two groups: The premaxilla agenesis-holoprosencephaly syndrome and frontonasal-median cleft syndrome^[15]. Midline clefts arise due to incomplete merging of the median nasal prominences that form the inter-maxillary segment. The premaxilla agenesis-holoprosencephaly syndrome (Demyer sequence) has a frontonasal deformity associated with hypotelorism, holoprosencephaly and facial deformity ranging from cyclopia to midline facial cleft with pre-maxillary ageneses. The median cleft face syndrome is often associated with a nasal deformity and hypertelorism usually either with no or little brain deformity (corpus callosum agenesis). In these cases

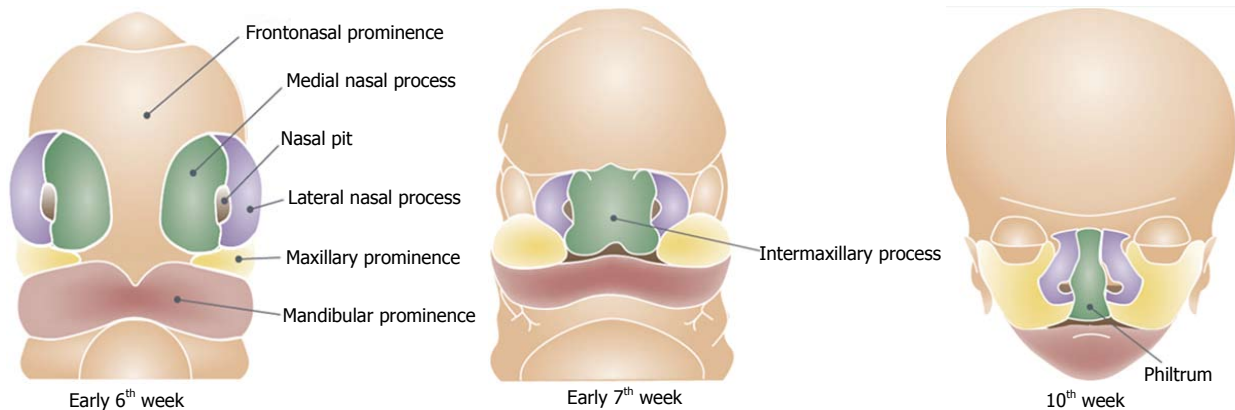


Figure 1 Development of the lip.

surgical reconstruction is feasible due to the probability of normal life expectancy.

DEVELOPMENT OF THE PALATE

Palatogenesis begins at the end of the 5th week and the development of the palate is not completed until the 12th week postconception^[11-13]. The palate develops from two primordia: The primary and the secondary palate. The most important cell types in palate development are the neural crest-derived palatal mesenchyme, ectoderm-derived epithelial lining and the most apical layer composed of periderm cells^[16]. The soft palate also includes the cranial paraxial mesoderm derived myogenic cells.

The primary palate is formed by merging of the medial nasal prominences during the 6th week and gives rise to the four central incisors and extends to the foramen incisivum.

The secondary palate that separates the nasal cavity from the oral cavity is the primordium of the hard and the soft palate and is formed by the fusion of neural crest mesenchyme that lies within the maxillary primordia. The development of the secondary palate starts with the outgrowth of two palatine shelves from the maxillary process that extend vertically on either side of the tongue. As the mandible grows downward and forward, the tongue's position descends. The palatal shelves subsequently rotate to a horizontal position dorsal to the tongue and then undergo intra-membranous ossification to form the palatine process of the maxilla and the palatine bone. The transition from vertical to horizontal position happens in the eight week postconception and is completed, incredibly, in only some hours. There is considerable sex difference in the timing of palatal closure. Shelf elevation and fusion begin a few days earlier in males than in females^[13]. Just as the formation of the lip, the subsequent fusion process is an incredibly complex process. Before fusion the palatal shelves are two cell layers thick. The outer layer is sloughed off (by apoptosis), leaving only a basal epithelial layer which composes the medial edge of each palatal shelf. The shelves grow towards

each other in the midline and approximate to form the midline epithelial seam. The seam subsequently degenerates, leading to mesenchymal confluence between the two palatal shelves. The fusion process of the shelves starts immediately behind the foramen incisivum and extends dorsally to close the palate like a "zip" (Figure 2). At the same time fusion with the nasal septum and the primary palate occurs. Gradually, bone extends from the palatine process of the maxilla and the palatine bone into the palatal shelves to form the hard palate. The posterior parts do not become ossified and extend posteriorly and fuse to form the soft palate, including the uvula^[13].

If the fusion process of the palate which occurs between the 9th and 12th week of gestation, is disrupted by either genetic, mechanical or teratogenic factors, a cleft of secondary palate results. Because the secondary palate closes from the foramen incisivum in a posterior(dorsal) direction, it is not possible for the palate to be open just posterior to the foramen incisivum in the hard palate and then subsequently fuse again in the soft palate part. If the initial process of fusion is defect the rest of the fusion process will not take place. This means that an intact soft palate implies the presence of an intact hard palate. Only a handful of cases have been described where "fenestrations" have been found in the midline of the palate seam^[17]. These cases have been attributed to trauma and not due to a defective fusion process. Especially the submucous cleft could be vulnerable^[17]. However, some mouse models do suggest that initial contact is made in the middle-anterior region with fusion proceeding in both anterior and posterior directions^[17].

Abnormality of the mandible appears to have a related cause with CP. Hypoplasia of the mandible (micrognathia) interferes with descent of the tongue and positions the tongue superiorly between the two palatal shelves. This causes mechanical disruption of palatal closure and could result in a CP. Micrognathia could be associated with Robin sequence. This sequence or phenomenon consisting of a triad of micrognathia, glossoptosis and breathing problems often involves an associated CP^[18]. This condition could be associated

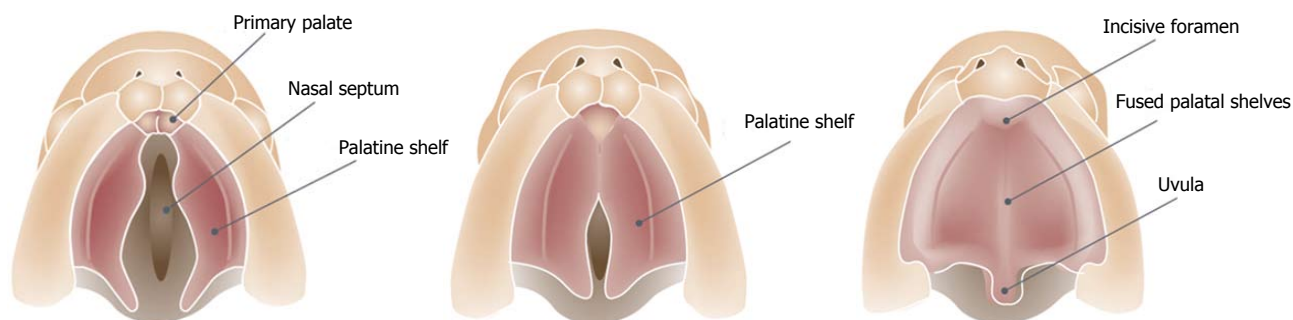


Figure 2 Caudal view of the fusion process of the palatal shelves.

with life threatening breathing problems postnatal and is often associated with other anomalies such as heart defects^[19].

IMPLICATIONS FOR DIAGNOSING AN OROFACIAL CLEFT

Fusion between the two maxillary processes differs on a molecular level from the fusion between the medial nasal process and the maxillary process. Together with the epidemiological differences this supports the view that CL/P (CL with or without CP) and isolated CP are two different entities^[6-13].

Postnatal the prevalence of associated anomalies is lowest in CL and ranges from 7.6%-41.4%^[6-13]. Data from postnatal studies show that concerning CLP the frequency is higher and ranges from 21.1%-61.2%. CP is the category most frequent associated with additional congenital anomalies and the prevalence of associated malformations with CP ranges from 22.2%-78.3%. Antenatally isolated CP is usually not diagnosed and associated abnormalities are reported in 39.1%-66.0% of fetuses with CLP^[20,21]. This high percentage probably reflects many different factors: The intra-uterine lethality of associated syndromes such as trisomy 13 or 18, pregnancy termination for severe malformations and the greater likelihood of diagnosing CL/P in the case of more associate abnormalities, especially if evaluation of the lip does not constitute part of routine screening.

It is becoming more frequent to use the trans-abdominal ultrasound screening during the second trimester of pregnancy to evaluate the face. Evaluation of the upper lip for possible CL/P is an optional element and has a sensitivity of 88% for detecting CL/P^[21]. However the overall sensitivity for OFCs is lower because the prenatal detection rate of CP is only 0%-1.4%^[21]. The very low detection rate of CP demonstrates that there are no satisfactory sonographic indicators of an isolated CP that might be one of the reasons why the palate is often not visualized during the ultrasound screening. Yet, the ultrasonographer should be aware that micrognathia could be associated with a CP. Subsequently, when the ultrasonographer encounters other malformations (such

as heart defects) with the micrognathia, evaluation of the palate is mandatory.

SPECTRUM OF OFCS

Some forms of CL only have a small indentation in the vermillion. This "forme fruste" with a small notch within the borders of the vermillion and a band of fibrous tissue running from the edge of the red lip to the nostril floor or a deformity of the nasal ala on the side of the notch, is unlikely be diagnosed by ultrasound. When CL is seen on 2D or 3D ultrasound, the position of the alar base (nostrils) can help to determine whether the alveolar ridge or palate is involved as well. An isolated incomplete CL without a maxillary or palatal defect will appear as a linear defect running from the lip towards the nasal floor (Figure 3A). In complete CL the lip defect could be well visualized, although the nose distortion is likely to be minimal (Figure 3B). In complete bilateral CL the maxilla is intact (there is no maxillary protrusion) and the alar bases or nostrils are symmetrical. Recently we have demonstrated that with a normal maxilla-nasion-maxilla angle, it was unlikely to find a cleft that included the alveolus^[22]. A complete CL with alveolar ridge involvement but without involvement of the primary palate is called an incomplete CL. Incomplete involvement of the alveolar ridge usually does not substantially change the position of the alar base. A complete CL is diagnosed as involvement of the complete lip, alveolar ridge and primary palate. A complete CLP includes the lip, alveolus, primary palate and whole secondary palate from foramen incisivum to include the uvula. In a complete CLP the lip involvement is relatively easy to detect by ultrasound, while the alar base is characteristically lateralized away from the cleft (Figures 3C and 4). In cases with bilateral CLP there is a protrusion of the maxillary process that is visible as an echogenic structure below the alar base (Figure 3D and E). This protrusion is not commonly seen in cases without a secondary CP and could help the ultrasonographer when confronted with a bilateral CL deformity. An important message for sonographers is that cleft alveolus does not necessarily equal CP. Lateralization of the alar base could help the

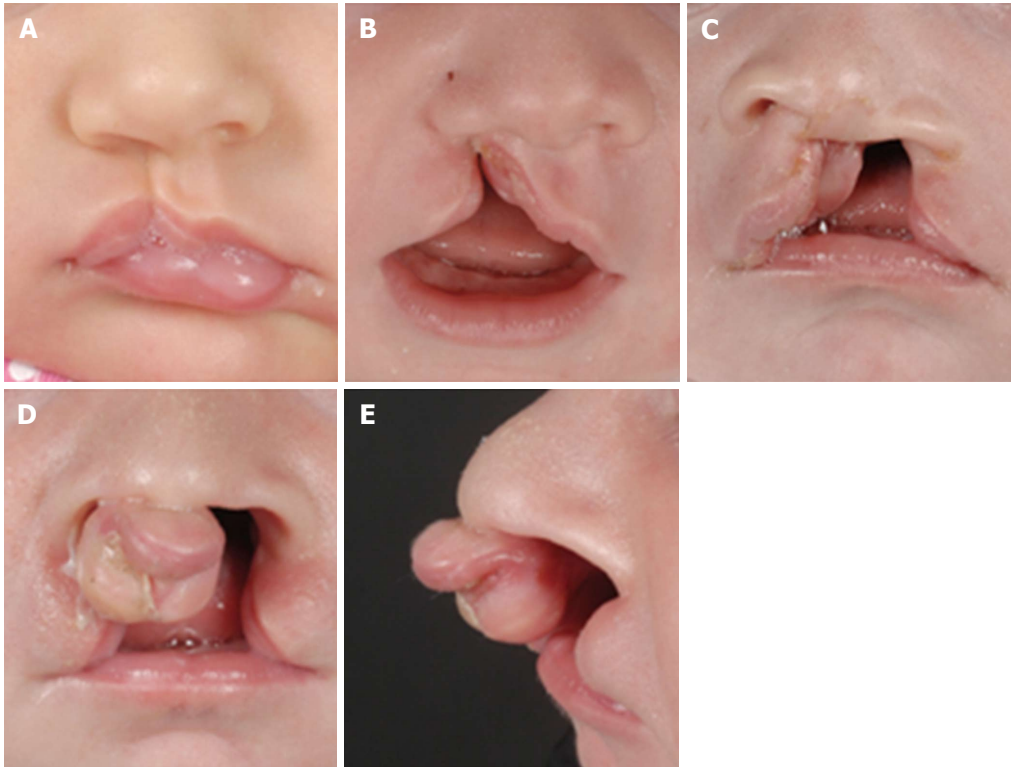


Figure 3 Different types of cleft lips. A: Unilateral microform cleft lip; B: Unilateral cleft lip and alveolus; C: Unilateral cleft lip and palate; D: Bilateral cleft lip and palate with protrusion of the intermaxillary process; E: Lateral view of bilateral cleft lip and palate.

ultrasonographer to diagnose complete CLP patients where the visualisation of the CP is difficult. Different techniques such as the oblique-face or flipped-face view make better 3-D visualization of the alar base^[8,9,10,23].

Unless other anomalies alert the ultrasonographer to the possibility, isolated CP is usually not diagnosed prenatally whereas the palate has a dome shaped structure and is surrounded by osseous structures making it difficult to visualize. The fact that the embryological fusion of the secondary palate starts from the foramen incisivum and proceeds dorsally is of importance during ultrasonography. An unremarkable uvula implies the presence of an intact palate. If the uvula is deformed or absent this might indicate a defect in the palate. The uvula can be visualized by ultrasound and the echo pattern of a normal uvula is typical and strongly resembles an "equals sign" (Figure 5)^[16]. It is important to realize that the vocal cords also have "two lines" and could resemble the "equal sign of the palate". However, the vocal cords are located lower than the soft palate. If the equals sign cannot be seen, CP cannot be ruled out and should be further examined by imaging the soft palate in a median sagittal section to visualize the palate structure in more detail. Larger prospective studies are needed to confirm this hypothesis. Implementation of the equals sign technique may improve the prenatal detection rate of isolated CP^[16,24]. Moreover if the ultrasonographer cannot visualise the middle part of the secondary palate (e.g., obstruction of the tongue) but the "equals

sign" is visible, it is suggestive of an intact palate. This could subsequently save the ultrasonographer time. However important techniques such as the "3D-reverse face" view technique^[8] and improved ultrasonography equipment and probes will also lead to better visualization of the palate. Martinez-Ten *et al.*^[9,23] described the importance of incorporating the "reverse-face view, with the flipped-face views and subsequently the oblique-face view into the algorithm of analysis a possible CP in the neonate. They also demonstrated that an accurate visualization of the palate required good initially acquired volume, fluid between the fetal tongue and palate, and curving of the plane to follow the structure of the palate^[23]. Subsequently, the oblique-face or flipped-face view makes better visualization in selected cases.

CLASSIFICATION

Orofacial clefting is multifactorial and etiologically heterogeneous. Therefore, proper classification is essential as different types of clefts may be variably associated with additional anomalies and chromosomal disorders. Over the years many different classification systems based on morphological, anatomical or etiological features of OFCs have been proposed. However, postnatal classification may not be applicable to prenatal findings.

An antenatal sonographic classification system has been proposed by Nyberg *et al.*^[25,26]. This classification



Figure 4 Prenatal, postnatal and post-surgery images of three different patients with cleft lip and alveolus, cleft lip and palate and bilateral cleft lip and palate respectively.



Figure 5 Echo pattern of a normal uvula visualized by ultrasound is typical and strongly resembles an “equals sign”.

shows four types of clefts and their relationship with the primary and secondary palate. Type 1: Isolated

CL alone, type 2: Unilateral CL and palate, type 3: Bilateral CL and palate and type 4: Median CL and palate. The so called type 5 clefts is another group of facial clefts associated with the amniotic band syndrome or the limb-body-wall complex and does not follow embryologic patterns but rather shows random types of often large and devastating defects^[25]. They further suggest that type 1 clefts are associated with a low rate of anomalies and type 2 and 3 clefts with intermediate prognosis. Type 4 and 5 clefts are universally associated with concurrent anomalies and with fatal outcome^[26]. The Nyberg classification has several shortcomings; CP for instance is not mentioned at all, while not all midline clefts have a fatal outcome.

A myriad of classification systems has been proposed and utilized over the years, however only a few have found clinical application. The most generally accepted classification was developed by Kernahan in

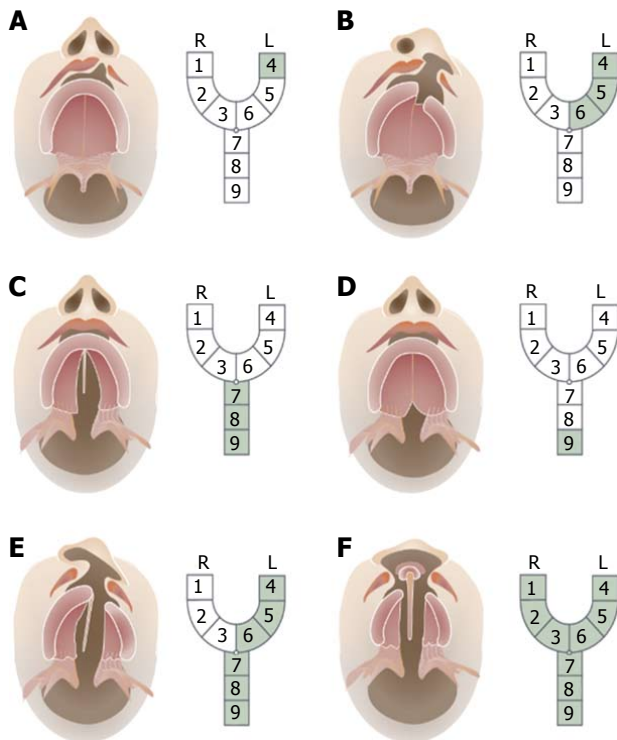


Figure 6 Kernahan's classification. The area affected by the cleft is labeled from 1-9, each of which represents a different anatomical structure: 1: Right lip; 2: Right alveolus; 3: Right premaxilla; 4: Left lip; 5: Left alveolus; 6: Left premaxilla; 7: Hard palate; 8: Soft palate; 9: Submucous cleft.

1971 (Figure 6), who proposed a striped Y-classification with the incisive foramen as the reference^[27]. This system is based on the resemblance of an intra-oral view of a CL and palate to the letter "Y". The area affected by the cleft is labeled from 1-9, each of which represents a different anatomical structure.

There has been a general move to adopt a simple classification system for clefts. The LAHSHAL code is often used as the preferred classification system by cleft surgeons ("L" = lip, "A" alveolus, "H" hard palate, "Soft palate"). It is compatible with ICD10 and allows clefts to be coded for computer use. The LAHSHAL codes split the relevant parts of the mouth in six parts and is written from the perspective of someone looking at the patient (*i.e.*, the first letter is for the patients right lip and the last letter for the patients left lip). The LAHSHAL code indicates for each patient whether there is a complete cleft (upper case letter, *e.g.*, "L") or an incomplete cleft (lower case, *e.g.*, "l") or no cleft. LAHSHAL would subsequently connote a complete cleft of the right lip/alveolus and incomplete cleft of the soft palate. SHAL would connote a complete left-sided unilateral CL, alveolus and palate. The midline Tessier clefts occur very infrequently, and are not included in these classifications.

CONCLUSION

Knowledge of the embryology of the face should add to the understanding and correctly diagnosing

OFCs. Failure of fusion between any of the facial swellings results in facial clefts and can occur either unilaterally or bilaterally and typically happens at the junction of the lateral incisor and the first premolar teeth. The depth of the cleft may vary from the soft tissue of the lip to a complete cleft of the maxillary bone. Lateralization of the alar base could help the ultrasonographer to diagnose a complete CLP patient where the visualisation of the CP is difficult. Maxillary protrusion seen with a bilateral CL is highly suggestive of a bilateral complete CL/palate. The embryological fusion of the palatal shelves starts anteriorly and proceeds posteriorly like a zip. An unremarkable uvula, visualised by ultrasound as an "equals sign", suggests an intact normal palate. If the ultrasonographer cannot visualise the middle part of the secondary palate, but the "equals sign" is visible, it is suggestive of an intact palate. This could save the ultrasonographer time.

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Pancreatic imaging: Current status of clinical practices and small animal studies

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Abstract

Different causative factors acting on the pancreas can result in diseases such as pancreatitis, diabetes and pancreatic tumors. The high incidence and mortality of pancreatic diseases have placed diagnostic imaging in a crucial position in daily clinical practice. In this mini-review article different pancreatic imaging techniques are discussed, from the standard clinical imaging modalities and state of the art clinical magnetic resonance imaging techniques to current situations in pre-clinical pancreatic imaging studies. In particular, the challenges of pre-clinical rodent pancreatic imaging are addressed, with both the image acquisition techniques and the post-processing methods for rodent pancreatic imaging elaborated.

Key words: Pancreatic imaging; Rats; State of the art clinical magnetic resonance imaging; 3.0T scanner; Quantitative magnetic resonance imaging

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Core tip: In this minireview, the challenges of pre-clinical rodent pancreatic imaging are addressed, basic clinical magnetic resonance imaging techniques and post-processing methods for rodent pancreatic imaging are also elaborated.

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INTRODUCTION

The pancreas is an important visceral organ performing both endocrine and exocrine functions. Abnormalities

of the pancreas result in diseases such as pancreatitis, diabetes, and pancreatic tumors^[1,2]. The onset of diabetes is usually long after beta cell dysfunction and insulin resistance^[3,4]; pancreatic cancer is generally asymptomatic and frequently diagnosed at a late stage^[5]; acute pancreatitis is a painful inflammatory condition often with severe complications and high mortality despite treatment^[6], while chronic pancreatitis can mimic the symptoms of pancreatic cancer and lead to misdiagnosis^[7]. The high incidence of pancreatitis and diabetes, and poor survival rate of pancreatic cancers have increased the demand for new diagnostic and therapeutic strategies^[8,9]. Herein multimodality multi-parametric imaging plays an indispensable role in disease detection, therapy guidance and patient follow-up. In this mini-review, current situations of common clinical practices and recent development of pre-clinical rodent studies in pancreatic imaging are inspected and discussed with the emphasis on basic magnetic resonance imaging (MRI) techniques and post-processing methods for rodent pancreatic studies.

OVERVIEW OF CLINICAL IMAGING MODALITIES

Ultrasound

As an initial step, abdominal ultrasound is most commonly used in screening for biliary stones and tumors, as this equipment is widely available at relatively low costs^[10]. However, the quality of ultrasound images and diagnostic accuracy are highly user-dependent, and the retroperitoneal location of the pancreas may impose image artifacts and hamper the ultrasound diagnosis^[11]. For further confirmation and staging of pancreatic diseases, imaging modalities with higher quality and sensitivity are needed.

Computed tomography

Contrast-enhanced multi-detector computed tomography (MDCT) remains the standard modality in clinic for the assessment of pancreatitis and pancreatic cancer^[12,13]. Due to its high spatial resolution and fast image acquisition, MDCT combined with contrast agents injection, has shown its powerful capacity in the staging of pancreatitis and pancreatic cancer with high sensitivity and specificity^[7,12].

MRI

MRI as a non-ionizing imaging modality has been increasingly utilized in clinic due to its multi-parametric capability^[14]. With the constant improvement of the new MRI hardware and imaging reconstruction algorithms, MRI is currently capable of acquiring images of spatial resolution approaching to that of CT. Meanwhile, with the application of accelerated parallel imaging techniques, most MRI protocols have the feasibility to be accomplished in one or a few breath-holds^[14,15].

Traditionally, T2-weighted MRI sequences are

commonly used to provide structural information on the anatomy of the pancreatic ductal system and lesions^[14]. MR cholangiopancreatography (MRCP) using heavily T2-weighted sequences has been widely applied as non-invasive alternative to endoscopic retrograde cholangiopancreatography (ERCP) for biliopancreatic duct system evaluation^[16]. The combination of dual-echo (in/opposed-phase) T1-weighted MRI sequences is useful for hemorrhage and fat content assessment^[14]. Dynamic contrast-enhanced (DCE) MRI scans are performed for differential diagnosis and grading of solid pancreatic lesions and pancreatitis by analyzing the pharmacokinetic parameters or contrast concentration curves^[17,18]. In addition, diffusion-weighted MRI (DWI) protocols have also shown a great potential to depict and characterize pancreatic diseases including acute/chronic pancreatitis and benign or malignant tumors^[19] without a need to use contrast agents.

Other more advanced but less popular pancreatic imaging modalities, often with a certain invasiveness or radiation exposure, include endoscopic and contrast enhanced US (EUS and CEUS), positron emission computed tomography and single-photon emission computed tomography incorporated with X-ray based CT (PET/CT and SPECT/CT) for improved spatial resolution and co-localization of imaging findings, etc. For a more comprehensive overview, a recent review article about imaging pancreatic diseases is recommended^[5].

CURRENT STATUS OF RODENT PANCREATIC IMAGING

Challenges in rodent pancreatic imaging

In order to develop new diagnostic and therapeutic strategies against pancreatic diseases, rodent models are commonly used in preclinical studies. However, imaging the pancreas in rodents proves to be extremely challenging due to motion artifacts and uncertain anatomy. The pancreas in humans represents a retroperitoneal solid organ, which can be identified by imaging modalities even without contrast enhancement, as stated above. However, unlike the human pancreas, the rodent pancreas appears as a soft, diffuse and irregularly lobulated organ, which is very difficult to discern from surrounding tissues^[19-22], even during open abdominal surgery (Figure 1). Pancreas-specific contrast agents would facilitate pancreas visualization, but currently those pancreatic specific markers are unavailable yet. Without specific labeling, rodent caudate liver lobes and abdominal fat tissue are frequently identified as pancreas by mistake. In some animal studies, pancreas associated injuries in other solid organs, instead of the pancreas itself, were investigated using contrast-enhanced protocols and MR spectroscopy (MRS)^[23,24].

Three dimensional pancreatic imaging

To avoid the misdiagnosis and to have a detailed

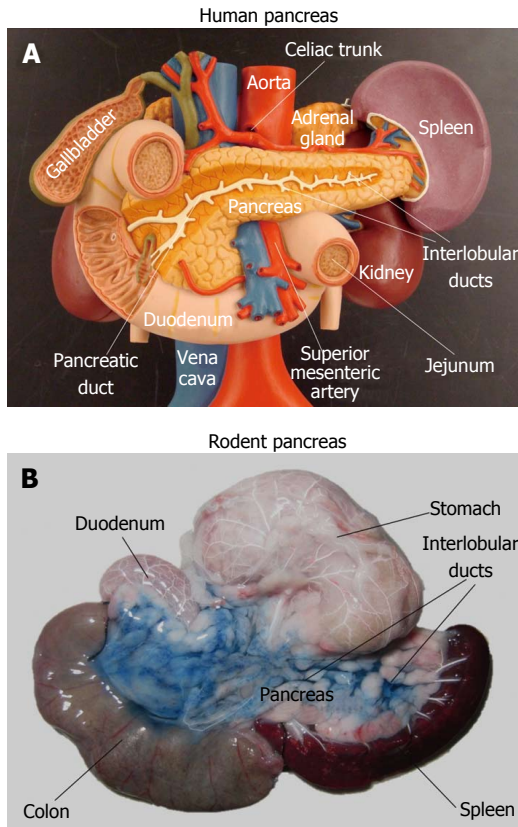


Figure 1 Anatomical difference between human and rodent pancreas. Unlike the human pancreas which is a well-defined solid organ (A), the rat pancreas appears as a soft, diffuse and irregularly lobulated organ (B), which is very difficult to discern from surrounding tissues even at open abdominal surgery. To better visualize it, the pancreatic ductal system was infused with Evans blue dye while the arterial system was injected with a barium sulphate suspension.

overview of the pancreas anatomy, two pancreatic imaging studies were performed using contrast-enhanced high-resolution three dimensional (3D) modalities to provide more precise anatomical information of the rodent pancreas^[20,22]. In a micro-CT study^[20], the *in vivo* rat pancreatic tail portion could be identified after a two-step contrast injection. Unlike the human pancreas that can be readily depicted by MRI even without using any contrast agent (Figure 2), detailed 3D rodent pancreatic anatomy and surrounding landmarks could only be demonstrated (Figure 2) by biliopancreatic local infusion of mixed contrast media in a post-mortem model^[22].

Diabetes imaging

In order to achieve early diagnosis in diabetes, the development of pancreatic specific contrast agents became a hot topic. Among others, some contrast agents were used to target pancreatic beta cells for diabetes related research subjects, for instance, glucagon-like peptide-1 (GLP1) receptor and GLP1 analogues have been frequently studied in rodent diabetes imaging^[25,26]. The micro-vascular changes in case of diabetes and pancreatic inflammation were

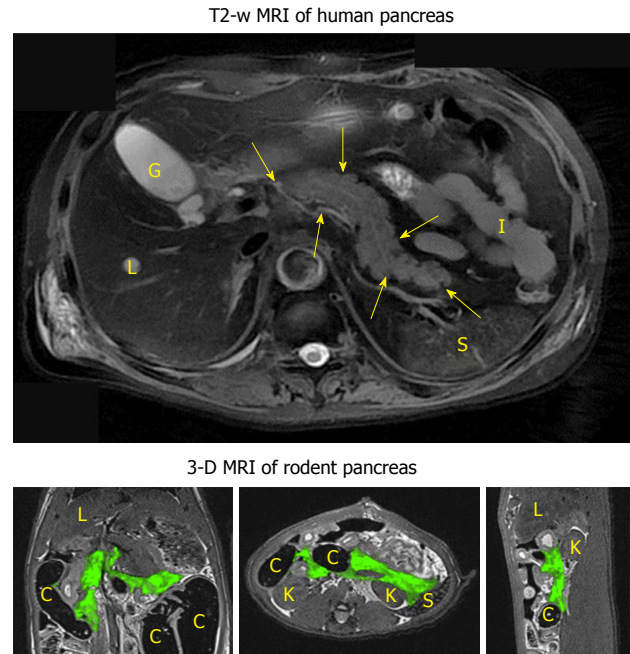


Figure 2 Typical human pancreatic magnetic resonance imaging vs rodent pancreatic magnetic resonance imaging. The upper transverse image shows the human pancreas (contoured by arrowheads) as a solid organ adjacent to the liver (L), gallbladder (G), spleen (S), kidney (K) and small intestines (I). The lower 3-D images display the coronal (left), transverse (mid) and sagittal (right) views of the contrast-infused rat pancreas with green color coding, adjacent to the liver (L), spleen (S), kidney (K) and colon (C). MRI: Magnetic resonance imaging.

also investigated^[27,28].

Pancreatitis imaging

The first attempt of rodent pancreatitis imaging started during the 1980's, conducted by Paaanen *et al.*^[29], in which Gd-DTPA was applied as a contrast agent for T2/T1 relaxation measurements in an acute hemorrhagic pancreatitis model. In 1989, Kushnir *et al.*^[30] performed several MRS experiments to identify imaging biomarkers in an acute pancreatitis model. More recently, specific nanoparticles were developed for pancreatitis imaging, with lipase as the target^[31]. Imaging of acute edematous pancreatitis can also be performed with MRCP, T2 relaxation measurement and non-specific contrast enhancement using modified protocols on a state of the art clinical MRI scanner^[32].

Pancreatic tumor imaging

The direct visualization of rodent pancreas and corresponding pancreatic landmarks could facilitate more precise diagnosis of a pancreatic tumor in the early stages. As a tumor grows to a certain volume, the identification of the solid tumor mass is much easier to perform than imaging other pancreatic disorders. Quantitative T2 and T1 relaxation measurements, DWI parameters and perfusion information can be obtained using multi-parametric MRI^[33]. Currently, rodent pancreatic tumor models are increasingly used

to investigate new therapeutic strategies in longitudinal follow-up studies by non-invasive MRI.

RODENT PANCREATIC IMAGING USING A CLINICAL MRI SCANNER

Rationale behind the use of a clinical MRI scanner for rodent pancreatic research

Due to the small size of the rodent pancreas, it is necessary to use high resolution 3D anatomical images for precise pancreas localization. Misdiagnosis could be avoided by carefully tracking the anatomy of the surrounding organs or tissues in 3D mode. Unfortunately, 3D anatomical MRI in the abdominal region is extremely challenging in commonly used high-field pre-clinical scanners, due to their high sensitivity to motion artifacts at high magnetic field and unavoidable long scanning durations. Alternatively, by the combined use of dedicated multi-channel coils and accelerated parallel imaging, clinical MR scanners have shown the feasibility and flexibility for rodent abdominal imaging^[22,32,33].

Limits and benefits of clinical scanners

The biggest problem using clinical scanners for small animal studies is the limited gradient strength^[34]. Most clinical MRI scanners have a gradient amplitude of 40 mT/m and slew rates up to 200 T/m per second. Although the maximum gradient amplitude in the recent 3T Siemens Prisma scanner has been increased to 80 mT/m, the gradient strengths are still up to 10 times lower than that of the current state of the art pre-clinical scanners. Consequently, the minimum slice thickness and minimal field of view (FOV) in the clinical systems are more restricted. In our studies, to maintain enough signal-to-noise-ratio (SNR), most 2D scans were performed with a slice thickness of 2 mm, which is identical to those acquired from small animal scanners. However, the minimal FOV is usually limited to around 70 mm. The limited gradient strengths also hamper the use of echo planar imaging (EPI) due to the prolonged readout, which leads to severe image distortions.

On the other hand, current state of the art clinical MRI scanners provide an excellent hardware stability, higher field homogeneity and a dedicated user interface, and are more widely accessible compared to small animal scanners. With the combination of dedicated clinical multi-channel surface coils and the self-calibrated parallel imaging techniques GRAPPA (GeneRalized Autocalibrating Partial Parallel Acquisition), it is possible to acquire high SNR images in rodent heterogeneous abdominal region with a sufficiently short scan duration. Moreover, lower magnetic field and application of GRAPPA provide a higher feasibility to rodent abdominal imaging.

Basic clinical MRI techniques for rodent pancreatic imaging

In our serial studies^[22,32,33], clinical scanners were used for pancreatic imaging: The Magnetom Tim Trio (Siemens, Erlangen, 45 mT/m, 200 T/m per second) combined with an 8-channel wrist coil; and the Magnetom Prisma (Siemens, Erlangen, 80 mT/m, 200 T/m per second) together with a 16-channel wrist coil. There are several standard clinical protocols which can be directly translated to pre-clinical research, including T2-weighted 3D turbo spin-echo (TSE) based SPACE (3D TSE with variable-flip-angle refocusing RF pulses) imaging and MRCP protocols, standard 2D multi-echo spin-echo sequences for T2 relaxation, as well as diffusion and perfusion sequences. The animal models introduced in these studies^[22,32,33] are a rat acute pancreatitis model and a rat orthotopic pancreatic tumor model, in which we intended to characterize pathological changes including edema, hemorrhage and necrosis by those modified MRI methods.

Three-dimensional volumetric images: As mentioned above, 3D imaging would facilitate the localization of rodent pancreas. The other benefit is the possibility of volumetric measurements in 3D. In case of edematous pancreatitis, edema volume is a biomarker for pancreatitis. Meanwhile, 3D views could also provide a more accurate measurement for irregularly shaped abdominal tumors (Figure 2). The volume of the target tissue can be obtained from post-process image segmentation. The most important 3D imaging protocols used here are T2-weighted SPACE and MRCP, which are also widely used in clinical MRI abdominal examinations.

Quantitative MRI measurements: Quantitative MRI relaxation measurements are useful in organ/tissue characterization. T2 mapping is helpful in the assessment of fluid content and hemorrhage; and native T1 mapping is essential for the calculation of the tissue concentration time curve (CTC) in DCE protocols. In these studies, mono-exponential T2 mapping and inversion recovery based T1 mapping were performed.

Measurements of Gaussian and non-Gaussian diffusion for water in biological tissues can be accomplished using DWI with different combinations of diffusion weightings. Mean diffusivity and diffusion kurtosis were obtained from 3-trace diffusion images.

Moreover, tissue perfusion can be characterized using DCE protocols, after the injection of a gadolinium based MRI contrast agent. After the signal conversion to gadolinium concentration using pre-contrast native T1 relaxation information, either semi-quantitative information or quantitative parameters from the pharmacokinetic Tofts model were extracted. Detailed MRI protocol parameters are elaborated in the different serial studies^[22,32,33].

Data processing

In these studies, open-source software and in-house built programs were used for data processing. This includes image segmentation, registration and 3D image visualization in open-source software ITK-SNAP (www.itksnap.org) and MeVisLab (MeVis Medical Solutions, Bremen, Germany); DICOM process, MRI mathematical modeling and quantitative analysis in Matlab programs (MathWorks, Natick, Massachusetts); and statistical analysis and data visualization using programs combining both Matlab and R (https://cran.r-project.org). Detailed image processing equations are included in the next section.

SOME OF THE ONGOING RESEARCH IN RODENT PANCREATIC MRI STUDIES

Identified objectives

Present research project aims at providing practical solutions to rodent pancreatic imaging using clinical facilities, from *ex vivo* to noninvasive *in vivo* imaging with the following systematic objectives identified: (1) To overcome the limitations of clinical MRI scanner for small rodent imaging studies; (2) detailed visualization of a complete pancreas and topographic landmarks through contrast enhanced CT and MR imaging in a rat postmortem model; (3) to explore noninvasive MR imaging methods for characterization of the Caerulein induced acute pancreatitis in rats; (4) to estimate the reliability of 3D isotropic MRI and quantitative multi-parametric MRI for characterization of an orthotopic pancreatic head tumor model in rats; and (5) to investigate therapeutic response of a vascular disruption agent in rat pancreatic tumor models with further modified quantitative multi-parametric methods.

Processing for quantitative parameters in rodent pancreatic MRI

MRI quantitative parameters can be obtained from advanced image processing methods using machine learning algorithms^[35-37]. For practical considerations, quantitative parameters are re-generated from in-house built Matlab programs using non-linear least square methods with CPU (Central Processing Unit) acceleration.

T2 mapping: Traditionally, the transverse relaxation time T2 is obtained using multi-echo spin-echo pulse sequences, by sampling signals at several different echo-times (TE), and fitted to either multi- or single-exponential decay functions^[38]. Fast T2 mapping can be obtained using balanced steady-state free precession (SSFP) readout^[39].

T1 mapping: On a clinical scanner, fast T1 mapping can be measured using inversion recovery methods or from variable flip angles experiments^[39,40]. Since the MRI acquisition has to be synchronized with

animal respiration, the effective repetition time (TR) is usually longer than 1 s. Thus, inversion recovery based protocols would be suggested for T1 mapping in rodent pancreatic imaging. Typically, the equation for measured signal in the inversion recovery T1 mapping experiment is a three-parameter function: $SI(t) = a + b \times \exp(-t/T1^*)$, where $SI(t)$ is the signal intensity after each inversion time t , and $T1^*$ is the effective longitudinal relaxation time. The actual T1 relaxation time can be obtained after correction for the flip angles^[41], or the Look-Locker readout^[42].

Diffusion-weighted model: In DWI experiments, the simple Gaussian diffusion can be assumed using a mono-exponential model. The two-compartment intravoxel incoherent motion model on the other hand is currently widely used in clinical pancreatitis and pancreatic tumor studies^[43,44], and separates diffusion into the true-diffusion and the pseudo-diffusion fraction. Alternatively, sampling with high b-values above 1000 s/mm² can be applied for non-Gaussian diffusion estimation using a diffusion kurtosis model^[45].

Post-processing for DCE model: The first step in DCE data post-processing is the conversion of the raw MRI signal to the tissue concentration time curve (CTC). The tissue concentration C_t of contrast agent (CA) during the DCE perfusion experiment is solved as: $1/T1(t) = 1/T1_0 + r1 \times C_t(t)$, where $T1_0$ is the T1 value before contrast injection, obtained from inversion recovery T1 mapping, and $r1$ is the longitudinal relaxivity of the applied CA. In a high temporal resolution DCE experiment, the T1 relaxation $T1(t)$ after CA injection can be converted from the signal intensity (SI) time curve as described previously^[33]. Alternatively, CTC information can be directly extracted from the dynamic T1 mapping.

The vascular input function (VIF) C_p is determined by the CA concentration in blood C_b : $C_p = C_b/(1 - Hct)$, which is obtained from CTC of the aorta or a major vein, and the hematocrit level Hct which is set to 42% in our studies. VIF is usually fitted into a bi-exponential function for further kinetic modeling. Perfusion indices, the transfer coefficient K_{trans} and the rate constant k_{ep} , can be obtained from the standard or the modified Tofts model^[46]. In practice, the discrete convolution can be constructed as a matrix multiplication. The fraction volume v_e of extravascular extracellular space is calculated as: $v_e = K_{trans}/k_{ep}$.

CONCLUSION

The diagnosis of pancreatic diseases and their management have been largely facilitated by ever advancing multimodal and multi-parametric imaging technologies in clinical settings. Likewise, thanks to the above-mentioned efforts, preclinical research on rodent models of pancreatic pathologies are also rapidly progressing in terms of visual identification

of rodent pancreas on 2D and 3D images, imaging characterization of common pancreatic disorders such as pancreatitis and pancreatic malignancies, and noninvasive imaging follow-up of investigative therapies for new drug development. Eventually clinical practices in patients suffering from those often deadly diseases on this complex visceral organ of pancreas will benefit from all these translational studies.

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Retrospective Study

Accuracy of crescent sign on ocular ultrasound in diagnosing papilledema

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Abstract

AIM

To study the usefulness of orbital ultrasonography in the diagnosis of papilledema.

METHODS

Fifty patients who were referred to the neurophthalmology clinic and clinically suspected to have papilledema were selected. Thorough, clinical examination with slitlamp biomicroscopy and visual acuity assessment was done. These patients underwent ultrasonography to demonstrate the crescent sign. The patients were further evaluated with the neurologist and magnetic resonance imaging (MRI) thus confirming the diagnosis of papilledema. The results of our ultrasonographic evaluation were correlated with final diagnosis after thorough clinical evaluation, imaging and the neurologist's opinion.

RESULTS

Out of 50 patients diagnosed having papilledema on MRI, 46 (92%) showed crescent sign on B scan ultrasonography. Headache was most common presenting complaint in 47 (94%) and idiopathic intracranial hypertension was most common underlying cause of papilledema in 30 (60%) cases.

CONCLUSION

"Crescent sign" seen on ultrasonography is a sensitive tool for diagnosis of papilledema. It is cost effective, less cumbersome and effective tool to differentiate

between papilledema and pseudo papilledema before subjecting the patients to costly investigations like MRI. A positive crescent sign should always be followed by MRI to find out the cause of papilledema.

Key words: Papilledema; Orbital ultrasound; Doughnut sign; Crescent sign; B scan

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Core tip: It is a retrospective study of 50 clinically diagnosed cases of papilledema where 46 cases showed a positive crescent sign on ocular ultrasonography. With an accuracy of 92%, ocular ultrasonography could be a cheaper and useful tool to confirm papilledema before subjecting these patients for MRI scans.

Bhosale A, Shah VM, Shah PK. Accuracy of crescent sign on ocular ultrasound in diagnosing papilledema. *World J Methodol* 2017; 7(3): 108-111 Available from: URL: <http://www.wjgnet.com/2222-0682/full/v7/i3/108.htm> DOI: <http://dx.doi.org/10.5662/wjm.v7.i3.108>

INTRODUCTION

Patients of papilledema have acquired bilateral, optic nerve head swelling due to increased intracranial pressure^[1]. Papilledema is an important reason of referral of patients to neuro-ophthalmology clinic. Suspicion of papilledema warrants timely ascertaining of its diagnosis, evaluation and management^[2]. Differentiation between papilledema and pseudopapilledema is important as patients with papilledema need thorough work up whereas patients with pseudopapilledema often only need reassurance monitoring and follow-up. The presentation of papilledema patients is varied, some present with headache, transient visual blurring, etc. Pseudopapilledema might present with more severe and acute visual presentation. To differentiate these and confirmation of diagnosis is usually done by magnetic resonance imaging (MRI). MRI is a tedious, costly investigation. Ultrasonography is a safe, cost-effective, easily available, non-cumbersome modality for diagnosis of papilledema. There are very few studies done which demonstrate the value of ocular ultrasonography in diagnosis of papilledema. Some studies have shown increased optic nerve sheath width by orbital ultrasonography correlating well with the final diagnosis of papilledema or pseudo papilledema^[3-5]. However all these studies measured the optic nerve width ranging anywhere from 3 mm to 3.3 mm to 5.7 mm along with 30° test. Measuring the optic nerve width and 30° test is difficult and requires an experienced ultrasonographer. None of the studies emphasized on the presence of “crescent” or “doughnut” sign alone, which is easier to elicit

and does not require a very experienced or skilled ultrasonographer. Our study shows the effectiveness of eliciting the “crescent” sign on ocular ultrasonography in diagnosing papilledema, before subjecting them to costly investigations like MRI.

MATERIALS AND METHODS

This was a retrospective study done in our institute from August 2015 to July 2016. Fifty patients diagnosed to have papilledema clinically, were included in this study. For each patient age, sex, duration of complaints, headache if present, associated systemic conditions were recorded. After measuring the visual acuity, all patients were clinically examined first by torch light, followed by slit lamp examination, intraocular pressure recording by applanation tonometry and a dilated fundus examination using 90 diopter lens. Informed consent was taken from all patients. Ocular ultrasonography using a 10 MHz probe (Sonomed Escalon E-Z Scan B5500+, Wayne, PA, United States) was performed on all in a supine position. If fluid was seen around the optic nerve, within the sheath, then it was noted as “crescent” or “doughnut” sign positive and it indicated presence of papilledema (Figure 1). All patients were referred to a neurologist and all crescent sign positive cases were subjected to neuro imaging. SPSS version 16.0 software was used for the statistical analysis.

RESULTS

The average age of presentation was 30 years (range 6 to 60 years). There were 37 (74%) females and only 13 (26%) males. Headache was the most common presenting complaint seen in 47/50 (94%) cases. Transient visual loss and diplopia were other complaints in very few cases. The duration of symptoms ranged from four days to 4 years. The most common systemic association was hypertension and diabetes mellitus in 3 patients (6%) each. Seventy-six percent ($n = 38$) patients out of 50 had no systemic disease.

Out of the 50 diagnosed cases of papilledema 46 showed positive “crescent sign” on ultrasonography. Thus the sensitivity of orbital ultrasonography in diagnosing papilledema was 92% ($n = 46$). Neuro imaging was done in all these cases and the most common cause of papilledema on MRI was idiopathic intracranial hypertension (IIHT) in 30 cases (60%) followed by space occupying lesions (SOL) in 8 (16%). Of the SOL cases, four had tumours in posterior cranial fossa, one in frontal lobe, two had meningiomas and one had craniopharyngioma. Sinus thrombosis was seen in 9 cases (18%). The common sinuses involved were sagittal sinus, sigmoid sinus and transverse sinus. Two patients had Grade IV hypertension retinopathy associated papilledema while one patient had multilocular hydrocephalus post shunt surgery. All these three cases along with one case of IIHT had normal

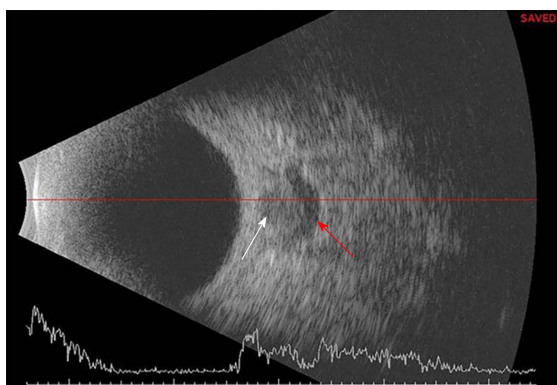


Figure 1 B scan ultrasound picture showing the normal round hypoechoic area of optic nerve (white arrow) with a hypoechoic crescent shaped area (red arrow) depicting “crescent” or “doughnut” sign.

ultrasonography (false negatives).

DISCUSSION

Papilledema is an important reason for emergency referral for patient to neurophthalmology clinic^[6]. However, sometimes it is difficult to differentiate papilledema from pseudo papilledema and patient has to be subjected MRI for confirmation of diagnosis^[7]. Cost, time duration, difficulties in claustrophobic and metal implants patients make it a tedious investigation. Ocular ultrasonographic measurement of optic disc width along with 30° test and presence of fluid around the optic nerve as a “crescent” or “doughnut” sign has been shown to be useful in confirming papilledema. However these tests, especially measurement of optic disc width and 30° test, require a cooperative patient with an experienced ultrasonographer and these tests are also time consuming. Eliciting the crescent sign alone is faster, has a shorter learning curve and can be done even by a less experienced ultrasonographer. In our study crescent sign was seen in 46/50 cases with a 92% sensitivity, which proves ultrasound B scan is a sensitive tool in initial diagnosis of papilledema. Sensitivity was 90%, 95% and 100% in studies by Neudorfer *et al.*^[3], Carter *et al.*^[4] and Mehrpour *et al.*^[5] respectively. However all these studies took a combination of measuring optic nerve width, 30° test and presence of crescent sign. Our study shows that presence of crescent sign alone has a good sensitivity of 92%. The advantage of eliciting the crescent sign is that it is faster and easier to perform compared to the other two tests. Four cases in our study had false negative scans. Of these two had grade IV hypertensive retinopathy (where we don't expect to have positive crescent sign), one case was with IIHT and another one had hydrocephalus post shunt surgery. In a single case by Sadda *et al.*^[8] the patient had bilateral disc edema with positive crescent sign on ocular ultrasonography but the patient was symptomless with normal intracranial pressure and

normal MRI. None of our cases showed false positivity.

Other investigative modalities like optical coherence tomography (OCT) and fluorescein angiography have also been studied in the diagnosis of papilledema^[9,10]. However, retro orbital anatomy cannot be imaged by OCT and fluorescein angiography has disadvantage of being invasive.

In conclusion, our study demonstrates, “crescent sign” on ultrasound for diagnosis of papilledema a highly sensitive tool in differentiating papilledema from pseudo papilledema before subjecting the patients to more costly, tedious and time consuming investigative modalities like MRI. A positive crescent sign should always be followed by MRI to find out the cause of papilledema.

COMMENTS

Background

Papilledema is a critical ocular sign which could be devastating and can lead to permanent disability. Confirmation of diagnosis is usually done by magnetic resonance imaging (MRI), which is a tedious, costly investigation.

Research frontiers

Ocular ultrasonography is a cost-effective, easily available, non-cumbersome, safe modality for diagnosis of papilledema.

Innovations and breakthroughs

There are very few studies done which demonstrate the value of ocular ultrasonography in diagnosis of papilledema. However all these studies measured the optic nerve width along with 30° test. Measuring the optic nerve width and 30° test is difficult and requires an experienced ultrasonographer. None of the studies emphasized on the presence of “crescent” or “doughnut” sign alone, which is easier to elicit and does not require a very experienced or skilled ultrasonographer. This study shows the effectiveness of eliciting the “crescent” sign on ocular ultrasonography in diagnosing papilledema.

Applications

The study results suggest that “crescent sign” seen on ocular ultrasonography is a sensitive and cost effective tool for diagnosis of papilledema.

Terminology

Papilledema is a serious condition of the eye which can have devastating complications. The infective organisms reach the ocular tissues via the blood stream. Ocular ultrasonography is readily available instrument with ophthalmologists. It is less cumbersome and can be quickly performed.

Peer-review

This study has valuable data that would be of interest if published.

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Predictive power of statistical significance

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Abstract

A statistically significant research finding should not be defined as a P -value of 0.05 or less, because this definition does not take into account study power. Statistical significance was originally defined by Fisher RA as a P -value of 0.05 or less. According to Fisher, any finding that is likely to occur by random variation no more than 1 in 20 times is considered significant. Neyman J and Pearson ES subsequently argued that Fisher's definition was incomplete. They proposed that statistical significance could only be determined by analyzing the chance of incorrectly considering a study finding was significant (a Type I error) or incorrectly considering a study finding was insignificant (a Type II error). Their definition of statistical significance is also incomplete because the error rates are considered separately, not together. A better definition of statistical significance is the positive predictive value of a P -value, which is equal to the power divided by the sum of power and the P -value. This definition is more complete and relevant than Fisher's or Neyman-Pearson's definitions, because it takes into account both concepts of statistical significance. Using this definition, a statistically significant finding requires a P -value of 0.05 or less when the power is at least 95%, and a P -value of 0.032 or less when the power is 60%. To achieve statistical significance, P -values must be adjusted downward as the study power decreases.

Key words: Statistical significance; Positive predictive value; Biostatistics; Clinical significance; Power

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Core tip: Statistical significance is currently defined as a P -value of 0.05 or less, however, this definition is inadequate because of the effect of study power. A better definition of statistical significance is based upon the P -value's positive predictive value. To achieve statistical significance using this definition, the power divided by the sum of power plus the P -value must be 95% or greater.

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INTRODUCTION

Scientific research has long utilized and accepted that a research finding is statistically significant if the likelihood of observing the statistical significance equates to $P < 0.05$. In other words, the result could be attributed to luck less than 1 in 20 times. If we are testing for example, effects of drug A on effect B, we could stratify groups into those receiving therapy vs those taking placebo vs no pharmacological intervention. If the data resulted in a P -value less than 0.05, under the generally accepted definition, this would suggest that our results are statistically significant. However, it could be equally argued that had it resulted in a P -value of 0.06, or just above the generally accepted cutoff of 0.05, it is still statistically significant, but to a slightly lesser degree - an index of statistical significance rather than a dichotomous yes or no. In that case, further testing may be indicated to validate the results but perhaps not enough evidence to outright conclude that the null hypothesis, drug A has no effect, is accurate in this sense.

The originator of this idea of a statistical threshold was the famous statistician R. A. Fisher who in his book *Statistical Methods for Research Workers*, first proposed hypothesis testing using an analysis of variance P value^[1]. In his words, the importance of statistical significance in biological investigation is to "prevent us being deceived by accidental occurrences" which are "not the causes we wish to study, or are trying to detect, but a combination of the many other circumstances which we can not control"^[2]. His argument was that $P \leq 0.05$ was a convenient level of standardization to hold researchers to, but that it is not a definitive rule as an arbitrary number. It is ultimately the responsibility of the investigator to evaluate the significance of their obtained data and P -value. For example, in some cases, a P -value of 0.05 may indicate further investigation is warranted while in others that may suffice.

There were however, opposing viewpoints to this idea, namely that of Neyman J and Pearson ES who argued for more for a "hypothesis testing" rather than "significance testing" as Fisher had postulated^[3]. Neyman and Pearson^[4] raised the question that Fisher failed to, namely that with data interpretation there may be not only a type I error, but a type II error (accepting the null hypothesis when it should in fact be rejected). They famously stated "Without hoping to know whether each separate hypothesis is true or false, we may search for rules to govern our behavior with regard to them, in following which we insure that, in the long run of experience, we shall not be too often wrong"^[4]. Part

of the Neyman-Pearson approach includes researchers assigning prior to an experiment, the alternative hypothesis which should be specific such that drug X has Y effect by 30%^[5]. This hypothesis is later accepted or rejected based on the P -value whose threshold was arbitrarily set at 0.05.

These two viewpoints between Neyman-Pearson and the more subjective view of Fisher were heavily debated and are ultimately recognized as either the Neyman-Pearson approach or the Fisher approach. In today's academic setting, the determination of statistical variance with a P -value has truly become dichotomous, either rejection or acceptance based on $P < 0.05$, rather than more of an index of suspicion as Fisher had originally proposed. However, an approach of confidence based on the P -value could be beneficial rather than a definitive decision based on an arbitrary cutoff.

The meaning and use of statistical significance as originally defined by Fisher RA, Jerzy Neyman and Egon Pearson has undergone little change in the almost 100 years since originally proposed. Statistical significance as original proposed by Fisher's P -value was the determination of whether or not a finding was unusual and worthy of further investigation. The Neyman-Pearson proposal was similar but slightly different. They proposed the concepts of alpha and beta with the alpha level representing the chance of erroneously thinking there is a significant finding (a Type I error) and the beta level representing the chance of erroneously thinking there is no significant finding (a Type II error) in the data observed^[6].

CLASSICAL STATISTICAL SIGNIFICANCE

Statistical significance as currently used represents the chance that the null hypothesis is not true as defined by the P -value. The classic definition of a statistically significant result is when the P -value is less than or equal to 0.05, meaning that there is at most a one in twenty chance that the test statistic found is due to normal variation of the null hypothesis^[2]. So when researchers state that their findings are "statistically significant" what they mean is that if in reality there was no difference between the groups studied, their findings would randomly occur at most only once out of twenty trials.

For example, consider an experiment in which there is no true difference between a placebo and an experimental drug. Because of normal random variation, a frequency distribution graph representing the difference between subjects taking a placebo compared with those taking the experimental drug typically forms a bell shaped curve^[7]. When there is no true difference between the placebo and the experimental drug, small differences will occur frequently and cluster around zero, the center of the peak of the curve. Relatively large differences will also occur, albeit infrequently, and these results are represented by the upper and lower tails of the graph. Assuming the entire area under the bell shaped curve equals 1, as represented in Figure 1,

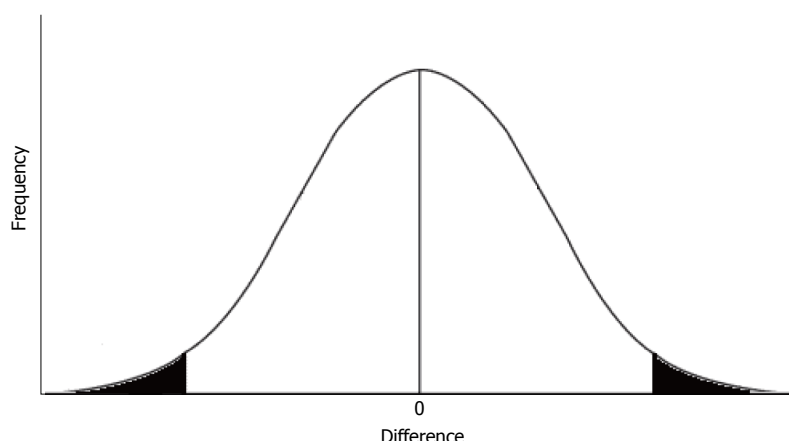


Figure 1 According to the classical definition, research findings are considered statistically significant when the difference observed falls in the upper or lower tails of the frequency distribution, represented above in black.

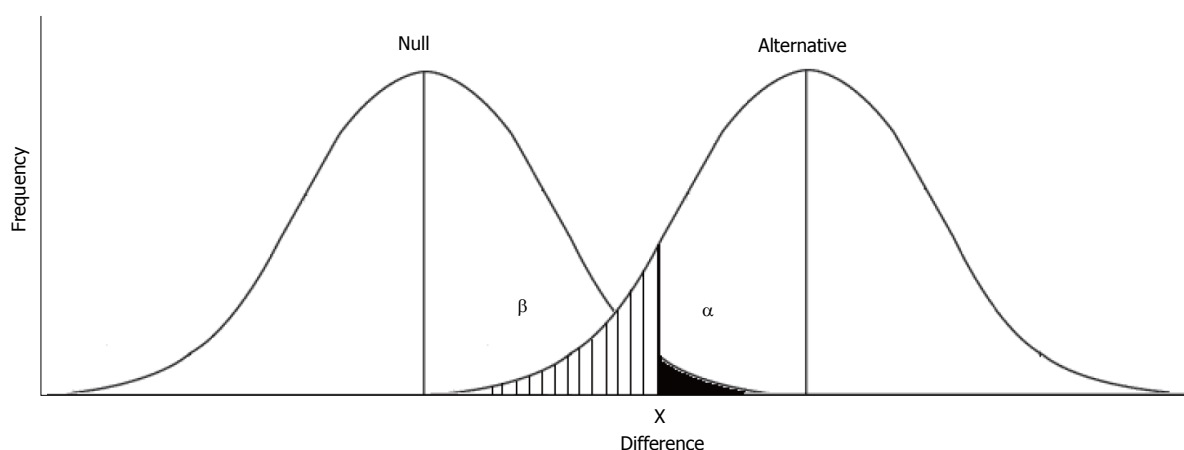


Figure 2 If the observed difference is greater than x , then we consider that the finding is statistically significant and the null hypothesis is rejected. If the difference found is less than x , then we accept the null hypothesis and reject the alternative hypothesis. The area in black represents a Type I error which occurs when the difference is greater than x , but the null hypothesis is in fact true. The lined area represents a Type II error which occurs when the difference found is less than x , but the alternative hypothesis is in fact true.

the findings are assumed to be statistically significant when the difference found falls in either the lower or upper 2.5% of the frequency distribution^[8].

Note that the classical definition of statistical significance according to Fisher relies only upon a single frequency distribution curve, representing the null hypothesis that no true difference exists between the two groups observed^[9]. Fisher's approach makes the primary assumption that only one group exists, as represented by a single frequency distribution curve, and P -values (the likelihood of a large difference being observed) define statistical significance. The Neyman-Pearson approach is slightly different, in that the primary assumption is that two groups exist, and two frequency distributions are necessary^[10]. In this approach, the tail of the frequency distribution representing the null hypothesis (no difference) is represented by alpha (α). Similar to the P -value, alpha represents the chance of rejecting the null hypothesis when in fact it is true, a Type I error^[11]. The tail of the frequency distribution representing the alternative hypothesis (a true difference

exists) is represented by beta (β). Beta represents the chance of rejecting the alternative hypothesis when in fact it is true, a Type II error. If we are doing a one-tailed comparison, e.g., when we assume the experimental drug will improve but not hurt patients, alpha and beta can be visualized in Figure 2. The area in black represents a Type I error and the lined area represents a Type II error.

A NEW DEFINITION OF STATISTICAL SIGNIFICANCE

It is time that the statistical significance be defined not just as the chance that the null hypothesis is not true (a low P -value), or the likelihood of error when accepting (α) or rejecting (β) the null hypothesis. While these statistics help us evaluate research data, they do not give us the odds of being right or wrong, which requires that we analyze both the P -value with β together^[12].

While it is helpful to visualize the concepts of alpha and beta on frequency distribution graphs, it is

Table 1 Statistically significant research findings can represent a true positive or false positive

Study findings	Reality	
	Alternative hypothesis true	Null hypothesis true
Significant P -value ≤ 0.05	True positive	False positive
Insignificant P -value > 0.05	False negative	True negative

Similarly, statistically insignificant findings may represent a true or false negative.

Table 2 When the P -value is utilized to determine whether or not a finding is statistically significant, 1-beta represents the sensitivity for identifying the alternative hypothesis, and 1-alpha represents the specificity

Study findings	Reality	
	Alternative hypothesis true	Null hypothesis true
Significant P -value ≤ 0.05	1 - beta (power)	Alpha (exact P -value)
Insignificant P -value > 0.05	Beta	1 - alpha

additionally illuminating to compare these concepts with sensitivity, specificity, and predictive values obtained from 2×2 contingency tables. In Table 1, the rows represent our statistical test results, and the columns represent what is actually true. Row 1 represents the situation when our data analysis results in a P -value of ≤ 0.05 , and row 2 represents the situation when our analysis results in a P -value of > 0.05 . The columns represent reality. Column 1 represents the situation when the alternative hypothesis is in reality true, and column 2 represents the situation when the null hypothesis in reality is true.

In Table 1, row 1 column 1 are the true positives because the P -value is ≤ 0.05 and the alternative hypothesis is true. Row 1 column 2 are false positives, because even though the P -value is ≤ 0.05 , the reality is that there is no significant difference and the null hypothesis is true. Similarly, row 2 column 1 are the false negatives because the P -value is insignificant ($P > 0.05$) but in reality the alternative hypothesis is true. Row 2 column 2 are the true negatives because the P -value is insignificant and the null hypothesis is true.

Table 2 shows our findings in terms of alpha and beta. In this case, alpha represents the exact P -value, not just whether or not the P -value is ≤ 0.05 . Beta is not only the chance of a Type II error (a false negative), it is used to determine the study's power which is simply equal to 1 - beta. Table 3 shows the same information in another way, showing the situations in which our test of statistical significance, the P -value, is in fact correct or is in error.

When we know beta and alpha, or alternatively the P -value and power of the study, we can fill out

Table 3 A Type I error corresponds to 1-specificity and a Type II error corresponds to 1-sensitivity when study findings are determined to be significant or insignificant based upon the P -value

Study findings	Reality	
	Alternative hypothesis true	Null hypothesis true
Significant P -value ≤ 0.05	Correct	Type I error
Insignificant P -value > 0.05	Type II error	Correct

Table 4 This 2×2 contingency table shows the corresponding values for a research study where a study finding is determined to be significant based upon a P -value of 0.05 and when the study's power is 80%

Study findings	Reality	
	Alternative hypothesis true	Null hypothesis true
Significant P -value ≤ 0.05	0.8	0.05
Insignificant P -value > 0.05	0.2	0.95

the contingency table and answer our real question of how likely is it that our findings represent the truth. Statistical power, equal to 1 - beta, is typically set in advance to help determine sample size. A typical level recommended for power is 0.80^[13]. Table 4 is an example 2×2 contingency table in the which the study has a power of 0.80 and the analysis finds a statistically significant result of $P = 0.05$. In this situation, the sensitivity of the test statistic equals the power, or $0.8/(0.8 + 0.2)$. The specificity of the test statistic is 1 minus alpha, or $0.95/(0.05 + 0.95)$. Our positive predictive value is power divided by the sum of power and the exact P -value, or $0.80/(0.80 + 0.05)$. The negative predictive value is the specificity divided by the sum of the specificity and beta, or $0.95/(0.95 + 0.20)$.

To be 95% confident that the P -value represents a statistically significant result, the positive predictive value must be 95% or greater. In the standard situation where the study power is 0.80, a P -value of 0.42 or less is required to achieve this level of confidence. As shown in Table 5, a power of 0.95 is required for a P -value of 0.05 to indicate a 95% or greater confidence that the study's findings are statistically significant. If the power falls to 90%, a P -value of 0.047 or less is required to be 95% confident that the alternative hypothesis is true (*i.e.*, a 95% positive predictive value). If the power is only 60%, then a P -value of 0.032 or less is required to be 95% confident that the alternative hypothesis is true. To determine how likely a study's findings represent the truth, determine the positive predictive value (PPV) of the test statistic:

$$\text{PPV} = \text{power}/(\text{power} + P\text{-value})$$

To determine the required P -value to achieve a 95%

Table 5 *P*-values corrected for study power

Study power	<i>P</i> -value
0.95	0.05
0.9	0.047
0.85	0.045
0.8	0.042
0.75	0.039
0.7	0.037
0.65	0.034
0.6	0.032

PPV:

$$P\text{-value} = (\text{power} - 0.95 * \text{power})/0.95$$

In the situation where the *P*-value is greater than the cutoff values determined by the preceding method, it is helpful to determine just how confident we can be that the null hypothesis is correct. This simply entails calculating the negative predictive value of the test statistic:

$$NPV = (1 - \alpha)/(1 + \beta - \alpha)$$

Finally, using this method we can determine the overall accuracy of a research study. Prior to collecting and analyzing the research data, pre-set values are determined for power and a cutoff *P*-value for statistical significance. If we want to be 95% confident that a research study will correctly identify reality, a pre-set power of 95% along with a pre-set cutoff *P*-value of 0.05 is required. At a pre-set power of 90%, a pre-set cutoff *P*-value of 0.01 is required. When the pre-set power is 80% or less, the maximum confidence in the accuracy of the study findings is at most 90% even when a pre-set *P*-value cutoff is extremely low. To determine the maximum level of confidence a study can have at a specific level of power and cutoff *P*-value (α), calculate the accuracy:

$$\text{Accuracy} = (1 + \text{power} - \alpha)/2$$

CONCLUSION

Statistical significance has for too long been broadly defined as a *P*-value of 0.05 or less^[14]. Using the *P*-value alone can be misleading because its calculation does not take into account the effect of study power upon the likelihood that the *P*-value represents normal variation or a true difference in study populations^[15]. If we want to be at least 95% confident that a research study has identified a true difference in study populations, the power must be at least 95%. If the power is lower, the required *P*-value to indicate a statistically significant result needs to be adjusted downward according to the formula $P\text{-value} = (\text{power} - 0.95 * \text{power})/0.95$. Furthermore, by using the positive predictive value of

the *P*-value, not just the *P*-value alone, researchers and readers are able to better understand the level of confidence they can have in the findings and better assess clinical relevance^[16]. Only when the power of a study is at least 95% does a *P*-value of 0.05 or less indicate a statistically significant result.

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Shortness of breath in clinical practice: A case for left atrial function and exercise stress testing for a comprehensive diastolic heart failure workup

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Abstract

The symptom cluster of shortness of breath (SOB) contributes significantly to the outpatient workload of cardiology services. The workup of these patients includes blood chemistry and biomarkers, imaging and functional testing of the heart and lungs. A diagnosis of diastolic heart failure is inferred through the exclusion of systolic abnormalities, a normal pulmonary function test and normal hemoglobin, coupled with diastolic abnormalities on echocardiography. Differentiating confounders such as obesity or deconditioning in a patient with diastolic abnormalities is difficult. While the most recent guidelines provide more avenues for diagnosis, such as incorporating the left atrial size, little emphasis is given to understanding left atrial function, which contributes to at least 25% of diastolic left ventricular filling; additionally, exercise stress testing to elicit symptoms and test the dynamics of diastolic parameters, especially when access to the "gold standard" invasive tests is lacking, presents clinical translational gaps. It is thus important in diastolic heart failure work up to understand left atrial mechanics

and the role of exercise testing to build a comprehensive argument for the diagnosis of diastolic heart failure in a patient presenting with SOB.

Key words: Diastolic heart failure; Exercise stress test; Left atrium; Shortness of breath; Work-up

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Core tip: Shortness of breath is a common clinical complaint. Etiologies such as systolic heart failure, obstructive airways disease or anemia have clear and reproducible physiological changes detectable through routine diagnostic tests. Diastolic heart failure (DHF) is often a diagnosis of exclusion. In the absence of directly demonstrating an elevation of left ventricular end diastolic pressures at rest or exercise, DHF is inferred by a combination of symptoms and resting echocardiography findings. We discuss the importance of a wider consideration, *e.g.*, left atrium function and exercise stress testing, in DHF work-up.

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INTRODUCTION

Most cardiological services are faced with a large number of referrals to diagnose and manage the symptom cluster of dyspnea or shortness of breath (SOB). Broadly the etiologies can be cardiac, respiratory, haematological, due to obesity or physical deconditioning. When a cardiac cause is considered likely, imaging modalities such as echocardiography and occasionally cardiac magnetic resonance imaging can rule out systolic heart failure or heart failure with reduced ejection fraction (SHF/HFrEF). Diastolic heart failure or heart failure with preserved ejection fraction (DHF/HFpEF) can be inferred, but requires greater analysis. Exercise stress protocols are also receiving greater attention for diagnosis of HFpEF.

To understand the controversies in DHF it is important to go back to the basics. HF is defined as "a clinical syndrome characterized by typical symptoms (*e.g.*, breathlessness, ankle swelling and fatigue) that may be accompanied by signs (*e.g.*, elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress"^[1]. From this, four points are important in the work-up of patients suspected with DHF syndrome: (1) Chronic functional SOB, is the main reason for seeking medical care, however asymptomatic structural changes can also be detected. The correlation of changes

at rest and with exercise with or without symptoms are yet to be adequately clarified; (2) in presentations with acute SOB admissions, risk stratifying diastolic abnormalities to a clinical course is also problematic^[2,3]; for example are the observed changes age related or evidence of diastolic dysfunction contributing to DHF; (3) three conditions must be satisfied to rule in the diagnosis of HFpEF: Clinical symptoms of heart failure; normal or mildly abnormal systolic function [left ventricular ejection fraction (LVEF) > 50%]; and demonstration of diastolic abnormalities in left ventricular (LV) relaxation and filling, and stiffness manifesting as increased LV filling pressures (invasively measured as LV end diastolic pressure > 16 mmHg (LVEDP) or mean pulmonary capillary wedge pressure or mean left atrial (LA) pressure > 12 mmHg), at rest or with exercise^[2,4]; and (4) demonstrating altered LV pathophysiology in the "resting state" are better established, while evaluation of dynamic diastolic changes (*i.e.*, during exercise) and alterations in left atrium (LA) metrics (*i.e.*, volume or function parameters), have not been given enough emphasis.

The incidence of HFpEF appears to be increasing relative to HFrEF. Combined data among HF presentations reveals an average prevalence of 54% (range 40%-71%)^[5]. The etiology and pathophysiological basis also appears different. Patients tend to be older with greater burden of co-morbidities^[6,7]. Cardiovascular and non-cardiovascular mortality is increased, although lower than HFrEF. However, survival trends are improving with HFpEF but not HFrEF^[8-11]. There have been numerous publications and guideline updates that provide a synopsis of pathophysiology^[12-14], clinical correlation and pathways for assessment of DHF^[1-3,15] and management^[16]. This review is focused on establishing the importance of LA function and exercise testing in the workup of a patient presenting with SOB. We also explore the rationale for including LA metrics under the umbrella of the DHF syndrome focusing on published work using echocardiography as the imaging modality (DHF and HFpEF are used interchangeably, where DHF is used in context of the syndrome and HFpEF in the scientific commentary).

LEFT ATRIAL ANATOMY, PHYSIOLOGY AND FUNCTION IN HEALTH AND DISEASE

The LA is predominately composed of overlapping and varying aligned layers of muscle fibers that have marked variation in thickness but is overall, significantly thinner than the LV. The left coronary artery and oblique vein, which drain into the coronary sinus, are the main arterial and venous blood vessels. Specifics on LA anatomy have been previously detailed^[17,18]. The LA has four important mechanical functions across three phases (Figure 1): (1) A reservoir (phase) to receive blood and store kinetic energy (as pressure) for LV filling that coincides with mitral valve closure to opening

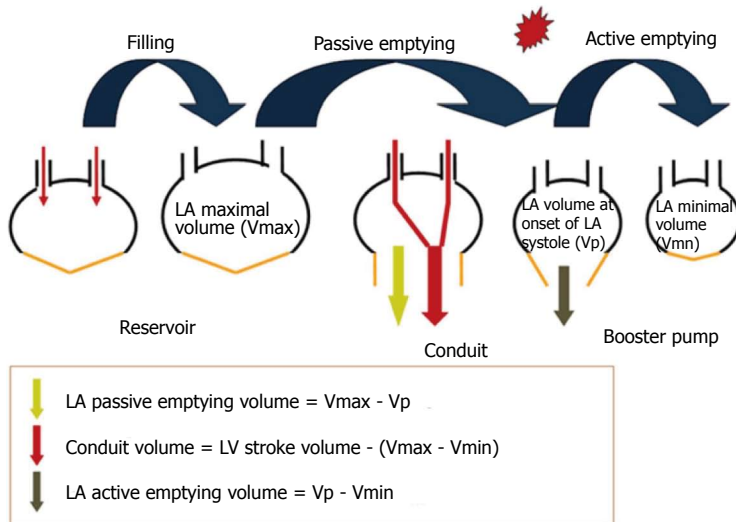


Figure 1 Phases of left atrial function. Left atrial preload is determined by blood flowing from the pulmonary vein. In this initial filling phase the LA acts a reservoir storing blood during left ventricular systole against a closed mitral valve. During LV diastole, diastasis and as the mitral valve opens it acts as a conduit, passively using stored energy to empty into the LV. Finally, in LV end diastole the LA contracts and actively empties blood completing the LV filling cycle. Reprinted from Karayannis *et al*^[21], with permission of the publisher (Copyright © 2007, Springer Science + Business Media. Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation). LA: Left atrial; LV: Left ventricle; Vp: Left atrial volume before atrial contraction; Vmax: Maximal volume (as defined at left ventricular end-systolic phase); Vmin: Left atrial minimal volume (as defined at left ventricular end-diastolic phase).

and ventricular events of isovolumic contraction, ejection and isovolumic relaxation; (2) a conduit (phase) for transiting blood (in early diastole) from the pulmonary veins to the LV after a pressure gradient develops to open the mitral valve and also passively during diastasis and is dependent on LV relaxation and preload; (3) a pump (contractile phase) to provide a “booster” depending on the preload, afterload, intrinsic contractility and electromechanical coupling [term defines the time between atrial electrical activation and mechanical activation (19)] to augment LV filling in late diastole; and (4) a suction effect to refill itself in early systole.

The LA contributes upto 30% of LV filling (The three phases can contribute around 40%, 35% and 25% respectively). LA flow from the pulmonary veins is continuous while LV filling is intermittent. The LA also acts as a volume sensor and regulates fluid balance by, neurohormonal function with production and regulation of natriuretic peptides, by regulatory (barometer) function *via* mechanoreceptors, and by interaction with renin angiotension aldosterone system/pathway (RAAS)^[19-25].

LV and diastole

LV diastole coincides with LA systolic phase. Of the four parameters used to define diastolic function, three, LV relaxation, distensibility (restoring force) and stiffness (compliance) are predominately determined by LV characteristics and morphology. The fourth, LV filling or preload has significant LA contribution and is a compensation to maintain stroke volume (SV). Through LA and LV preload, afterload, contractility and electromechanical coupling passive and active atrioventricular connectivity are established. There

are several publications that describe and evaluate LV aspects of DHF are cited^[1,3,12,13,19,26,27]. Diastole is described in four phases and these phases can be related to phasic LA events (Figure 2)^[28]: (1) Isovolumic relaxation during LA reservoir period; (2) rapid early filling; (3) diastasis during LA conduit phase; and (4) late filling, during atrial contraction phase.

Left atrial remodelling

When there is pressure and volume overload the process of atrial remodeling starts. In 220 healthy patients, age related LA indexed volumes changed only beyond the eight-decade^[29]. In contrast, and without increasing LA size, changes in phasic atrial volumes and augmentation of LA contraction occur earlier, corresponding with age related alterations in LV diastolic relaxation^[30,31]. Changes in the indexed LA volume (LAVi) appear to parallel the grade of diastolic dysfunction (DD)^[22]. Atrial arrhythmias is an independent precipitant of atrial remodeling. The response of atrial cell to external stress incites hypertrophy, fibrosis and subsequently LA dilatation and hypocontractility^[21]. LA dysfunction may alter the reservoir and conduit functions of the atrium and reduce the ability to absorb increases in LVEDP being transmitted to the pulmonary vasculature, for which there is a threshold similar to LV Frank-Starling mechanics^[32]. Loss of phasic LA pump function can also lead to symptoms by reducing late LV diastolic filling, which is more marked when there is preexisting systolic impairment^[25,33]. Pressure load to the LA can be seen in mitral stenosis and or increased LVEDP. Volume loading occurs in mitral regurgitation, intracardiac shunts or arteriovenous fistulae and high cardiac output states. These have to be factored in using LA metric when

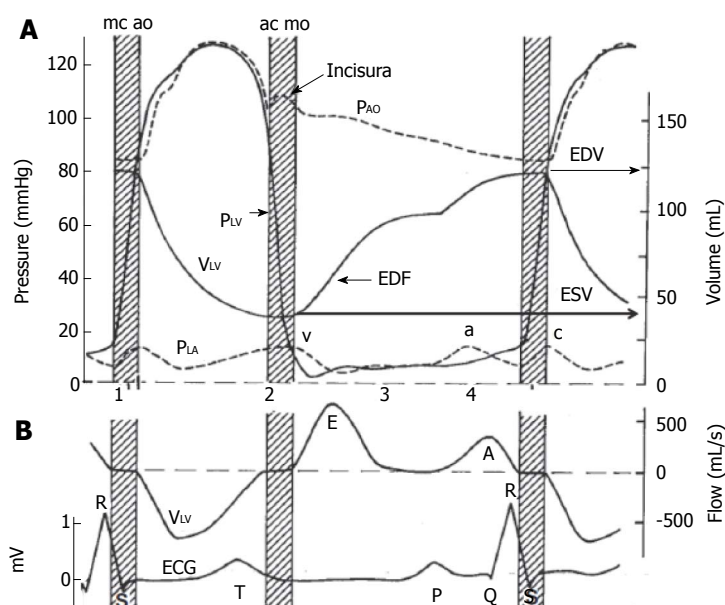


Figure 2 Describes the volume and flow relationships in the left atrium and left ventricle throughout one cardiac cycle, *i.e.*, systole and diastole. A: Pressure (P) and volume (V) are presented for the Aorta (Ao), left atrium (LA) and left ventricle (LV). Systole: During the early phase between mitral valve closure (mc) and aortic valve opening (ao) is the isovolumic contraction phase (stripped bar), where there is increase in PLV (solid line) without change in VLV (solid line). This is followed by ventricular contraction with a rise in PLV and PAO (upper dashed line), that peaks mid cycle, and a reduction in VLV. Diastole: At the end of LV contraction, and when the PLV is lower than the aorta the aortic valve closes (ac), followed by a period of isovolumic LV relaxation (stripped bar), where there is reduction in PLV without a change in VLV. The Incisura or dirotic notch describes the small backflow of blood into the LV. Early diastolic point of early diastolic filling. In diastole PLA is generated early by the reservoir and conduit atrial function (v wave - lower dashed line) and corresponds with early diastolic filling (EDF) and a late atrial contraction or booster function (a wave) and contributes to late diastolic filling. Ventricular volumes are as end diastolic or end systolic (EDV or ESV; solid line). Cardiac sounds are shown as 1-4; B: Diagram showing relationship between electrical conduction and blood flow with an additional catheter in the LV. Systolic blood flow out of the ventricle (V LV), is followed by early diastolic blood flow into the LV (E wave), late blood flow into the LV during LA contraction (A wave). A standard ECG lead II shows LA depolarization, LV depolarization, and LV repolarization (P wave, QRS complex, and T wave, respectively) (Published in Ref 28, figure provided courtesy of Dr. John V. Tyberg and Dr. Henk E. D. J. ter Keurs. Permission required). ECG: Electrocardiogram.

evaluating DD.

ROLE OF ATRIUM IN DHF WORKUP

In a patient with SOB, echocardiography will firstly confirm LV systolic function (*i.e.*, normal or mildly impaired ventricle (LVEF > 50%). A body of evidence is developing however to suggest that "sub clinical" systolic dysfunction such as reduced longitudinal LV shortening are present, and occur before the alteration seen in LVEF. At this stage the clinical context for DHF is evolving. Cardiac imaging with echocardiography however does not directly measure LVEDP and infers this by changes in volume, blood and tissue velocities. Invasive measures (LV pressure tracing or pressure volume data) and natriuretic peptides can provide direct information on myocardial stretch and hence diastolic abnormalities^[15]. However, the noninvasively evaluated e/e' (ratio of early diastolic transmitral velocity to early diastolic tissue velocity) serves as a surrogate of LV EDP.

Some patients manifest symptoms during exercise and this similarly can be assessed^[27,34-37]. There is no single non-invasive index that confirms or rules out the diagnosis, however using a combination of parameters, this can be achieved (Figure 3). Furthermore it is unclear if any one parameter provides greater weight than another.

Left atrium as a biomarker

There is a volume of data to support LA enlargement and adverse cardiovascular outcomes independent of age, gender and the major comorbid cardiovascular risk factors^[22,38,39]. In fact LA dilatation should be considered pathological before the eighth decade^[28]. Among 2042 residents in Olmstead County, Minnesota over 45 years of age, LAVi predicted all cause mortality, as did the grade of DD^[40]. From the same community, retrospective analysis of 1160 participants (> 65 years) followed for 3.8 ± 2.7 years, LAVi > 32 mL/m² predicted risk for first cardiovascular event ($P = 0.003$)^[41]. Several studies with 851 and 1495 patients over 65 years of age, found that measures of LA size predicted new development of HF^[42,43]. This risk was also demonstrated in 483 younger participants (mean age 47 years) followed for 6.8 years, where Leung *et al* showed that LAVi > 24 mL/m² was the only independent echocardiographic predictor of cardiovascular death, congestive heart failure, myocardial infarction, stroke and atrial fibrillation. Using a variety of methods, studies show an increase in cardiac and all cause mortality in a general population^[44,45], following myocardial infarction^[46,47], and with dilated cardiomyopathy^[48]; predicts ischemic heart disease^[41,49,50], atrial fibrillation and stroke^[40,41,44,49-56].

Alteration in LA mechanics (function), with or without LA dilatation, correlate with disease states such as

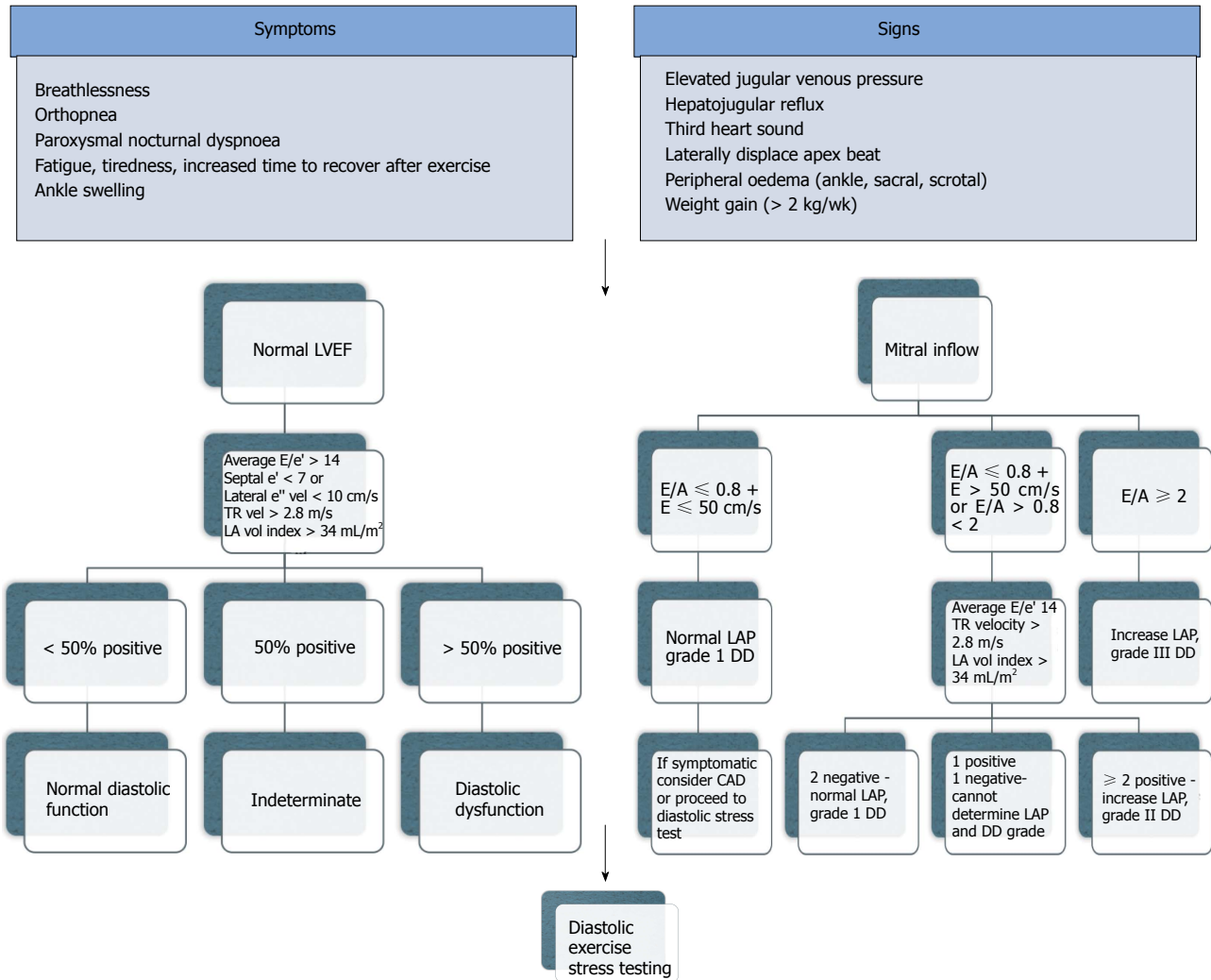


Figure 3 How to diagnose heart failure with preserved ejection fraction. From the 2016 consensus statements of HF, the diagnosis of HF requires 4 important factors: (1) the presence of symptoms and/or signs of HF; (2) a “preserved” EF (defined as LVEF $\geq 50\%$ or $40\%-49\%$ for HfmrEF); (3) elevated levels of natriuretic peptides (BNP > 35 pg/mL and/or NT-proBNP > 125 pg/mL); (4) objective evidence of other cardiac functional and structural alterations underlying HF; and (5) In case of uncertainty, a stress test or invasively measured elevated LV filling pressure may be needed to confirm the diagnosis. However in clinical practice many patients present predominately with a symptom such as SOB. The new guidelines are a positive step forward, and the authors for the first time acknowledged LA size, a surrogate for chronically elevated LVEDP and LA dysfunction. They fall short however as there are confounders for the abnormalities and none of the factors can be conclusively correlated to symptoms, where exercise testing could. A: Atrial filling velocity; BNP: Brain natriuretic peptides; E: Early filling velocity; e': Early mitral annular tissue doppler velocity; EF: Ejection fraction; HfmrEF: Heart failure mid-range ejection fraction; LA: Left atrium; LAP: Left atrial pressure; LV: Left ventricle; LVEDP: Left ventricular end diastolic pressure; NT-proBNP: N Terminal Brain Natriuretic peptide; TR: Tricuspid regurgitation (adapted from References 1 and 3).

hypertension, diabetes and renal impairment, and to adverse outcomes^[57,58]. In 1802 participants of the Dallas Heart study imaged with magnetic resonance imaging, decreasing LA emptying fraction was independently associated with mortality but not LAVi^[59]. In HF the reservoir and conduit functions are inversely related with Doppler parameters of DD and LVEDP. As HF progresses atrial contractility also gradually declines^[60-62]. Early changes in LA mechanics, correlations with comorbidities and disease severity and recovery with treatments, have been demonstrated for hypertension^[63-65], atrial fibrillation^[66-70] and valvular heart disease, using a variety of methods^[19,22].

Left atrium as a barometer

LA changes particularly the LAVi reflects the chronicity

and cumulative effects of changes in LV filling pressures. While the LAVi does not reflect acute changes in LV pressures it can be used as a barometer for chronically elevated LV filling pressures. This change can persist for some time after pressures have normalized. Increased LA volume can also be seen in athletes, bradycardia, anemia, high output states, atrial arrhythmias and mitral valve disease, independent of diastolic dysfunction. When these conditions are excluded LAVi > 34 mL/m² should alert treating physicians to the possibility of DD and raised LV filling pressures^[15].

To summarize the data, firstly LA size is a marker of health in a population; secondly a change in size highlights a remodeling process that predicts adverse outcomes; and thirdly alterations in LA size and mechanics potentially is caused by alterations in LV

diastolic filling abnormalities due to atrioventricular interdependence^[40].

IMAGING THE LEFT ATRIUM

Conventional echocardiography is sufficient to assess atrial size, but a combination of conventional and novel techniques are required to assess atrial mechanical functions.

Left atrial size assessment

M-mode and 2D echocardiography measuring the antero-posterior diameter, as performed in early studies, is now agreed to be an inadequate measure of LA size. Both the American and European Society of Echocardiography are in consensus that LAV using either the ellipsoid model or Simpson's method in two and four chamber apical views is more accurate as LA enlargement occurs asymmetrically. When the LAV is indexed (LAVi) it provides the strongest association, most sensitive predictor and risk stratification tool for cardiovascular outcomes^[2,3,22,38]. A detailed description of LAV is highlighted below^[55].

LA passive volumes consist of: (1) Preatrial contraction volume (V_{preA}), measured at the onset of the P-wave on an electrocardiogram (ECG); (2) minimal LA volume (V_{min}), measured at the closure of the mitral valve in end-diastole; and (3) maximal LA volume (V_{max}), measured just before the opening of the mitral valve in end-systole.

LA active volumes are: (1) LA reservoir volume ($V_{\text{max}} - V_{\text{min}}$); (2) LA conduit volume (LV total SV - LA reservoir volume); (3) LA passive emptying volume ($V_{\text{max}} - V_{\text{preA}}$); and (4) LA contractile volume ($V_{\text{preA}} - V_{\text{min}}$).

Physiological associations of LA size have been noted with body size and gender, but these differences are not apparent once indexed to BSA. Age related changes are seen at the extremes but not with normal aging. Based on the sensitivity and specificity for predicting cardiac events, population studies have shown mean LAVi by biplane Simpsons or area length method was between $20\text{--}23 \pm 6\text{--}7 \text{ mL/m}^2$, giving a normal value of $22 \pm 6 \text{ mL/m}^2$ ^[31,40,44,51,53]. In the guidelines, 1 standard deviation (SD) from the mean $> 28 \text{ mL/m}^2$ is considered LA enlargement and 2 SD from the mean $> 34 \text{ mL/m}^2$ for DD^[3,18,21]. Pressure load to the LA can be seen in mitral stenosis and or increased LVEDP. Volume loading occurs in mitral regurgitation, intracardiac shunts or arteriovenous fistulae and high cardiac output states. These have to be factored in evaluating DD and LA changes. Factoring these conditions LAVi has been shown to strongly correlate with the degree of DD and even differentiate between normal and pseudonormal filling patterns^[19,20,22,50,71,72].

Left atrial function assessment

The gold standard test atrial volume loop is invasive

and not routinely available. Four established echocardiographic parameters can provide information on the varying phases of LA function with advantages and disadvantages (Table 1).

2D volumetric analysis (the volume method) is the simplest but requires skill in obtaining the images and is time consuming. It uses LA volume at their maximum, minimum and just before LA systole to determine function.

Spectral (pulsed wave) Doppler of transmitral flow and pulmonary veins (sampled at mitral leaflet tips) are readily available, easy to use but only provides estimate of LA function. It is dependent on immediate loading conditions and can be affected by myocardial tethering acquisition angle, heart rates, atrial fibrillation, conduction system disease, age related reductions in LV diastolic compliance, altered hemodynamics and mitral valve disease. Peak transmitral A wave velocity, velocity time integral and atrial fraction can be used to measure LA contractile function and has been beneficial in following correction of atrial fibrillation with cardioversion, catheter ablation or surgery^[53,71-78]. The atrial ejection force can be calculated with several assumptions of the density of blood and a circular mitral annulus area, where diameter is measured in 4-chamber view. This has found correlation with return of atrial function post cardioversion, adverse cardiovascular remodeling and cardiovascular events^[79,80], although significant technical limitations persist^[20]. Importantly all Doppler measurements can only be performed in sinus rhythm.

Tissue Doppler imaging of intrinsic myocardial velocity (e.g., mitral annulus), can provide regional and when averaged from several sites, global function. It is a low-velocity and high amplitude signal and has the advantage of being load independent. Tissue Doppler has deficiencies of angle dependency (acquisition angle - long axis), is dependent on cardiac motion and myocardial properties such as tethering and annular sampling site. A' values has been shown to be a useful surrogate of global LA function, while all parameters (S', E' and A') provide useful prognostic information^[20,31,81-86].

Deformation analysis with strain and the speed of deformation with strain rate imaging can quantify regional and global function independent of tethering. Values however show regional variation^[63,73]. Positive values are seen with chamber dilatation and wall stretch and negative values with contraction. Similarly this method has shown correlations with clinical outcomes and prognosis such as maintenance of sinus rhythm and atrial mechanics in atrial fibrillation^[67-71,87], New York Heart Association Functional Class^[97], LA contractile function^[63,65], hypertensive heart disease^[64,66] and valvular heart disease^[19].

EXERCISE DIASTOLOGY

SOB and exercise intolerance due to HFpEF, should demonstrate an increased LVEDP with exercise. The

Table 1 Imaging modalities and their correlations with components of atrial function¹

LA function	Volumetric	Spectral Doppler			Tissue Doppler and deformation indexes		
	LA volume fraction	Transmitral flow	PV flow	Composite indexes	TDI	Strain (ε)	Strain rate
Global	LA EF [(LAmax - LAmin)/LAmax]	-	-	LAFI	-	-	-
Reservoir	Expansion index [(LAmax - LAmin)/LAmin]	-	S	-	S'	S; total	S
Conduit	Passive EF [(LAmax - LApre-A)/LAmax]	E E/A	D	-	E'	e-pos	E
Contractile (Booster)	Active EF [(LApre-A - LAmin)/LApre-A]	A E/A AFF	PVa	Ejection force (AEF) LAKE	A'	a-neg	A

¹Table modified from Ref^[20,22]. ε: Strain; A/A': Atrial contraction velocity/tissue Doppler velocity; AEF: Atrial ejection force Atrial ejection force = $0.5 \times 1.06 \text{ g/cm}^3 \times \text{mitral annulus area (peak A velocity)}$. Mass of blood is calculated as the product of the density of blood ($\rho = 1.06 \text{ g/cm}^3$) and volume of blood passing through mitral annulus; AFF: Atrial filling fraction, the ratio of the velocity time integral of the mitral A wave to the total diastolic transmitral flow; E/E': Early diastole velocity/tissue Doppler velocity; EF: Emptying fraction; LA: Left atrial; LAEF: Left atrial emptying fraction; LAFI: Left atrial functional index; LAKE: Left atrial kinetic energy; LAmax: Maximum left atrial volume; LAmin: Minimum left atrial volume; neg: Negative; pos: Positive; preA: Preatrial contraction; PV: Pulmonary venous; PVa: Pulmonary venous reversal velocity; S/S': Ventricular systole velocity/tissue Doppler velocity; TDI: Tissue Doppler imaging.

proven exercise protocols are stress echocardiography, combined stress echocardiography and cardiopulmonary stress test, and right heart catheterization with exercise^[27,34-36,88-98]. HFpEF is a systemic condition with an interaction of the primary cause coupled with secondary pathophysiological changes in the LV and LA. The continuity of the vasculature places the cardiac and peripheral endothelial beds at risk of injury when chronically exposed to risk factors. This loss of compliance or efficiency can see disproportionate rises in LV filling pressures, which can be buffered for, *e.g.*, by changes in atrial function^[27]. Thus a combination of deficits in arterial-ventricular-atrial function will be present in symptomatic individuals where a rise in LVEDP or LA pressure is the common denominator.

Burgess *et al*^[91] studied 37 patients at baseline and after supine cycle ergometry, and found that the e/e' of > 13 correlates with an elevated LVEDP during exercise. In another 166 patients post-exercise $e/e' > 13$ was highly specific (90%) for stratifying an exercise capacity of < 8 METs or > 8 METs^[91]. Nedeljkovic *et al*^[89] studied 87 patients with HTN, exertional SOB and normal resting LV function with combined exercise stress echocardiography cardiopulmonary testing to identify masked HFpEF found correlations between $e/e' > 15$ and reduced peak $\dot{V}O_2$ and other parameters with high sensitivity and specificity. Maeder *et al*^[36] identified 14 patients with diagnosed HFpEF and matched controls, who subsequently underwent supine cycle ergometer exercise, found that patients with HFpEF achieved a similar pulmonary capillary wedge pressure (PCWP) to asymptomatic controls at a much lower workload. However, contrary to Burgess *et al*^[91], the e/e' did not reflect the hemodynamic changes during exercise in HFNEF patients.

Pulmonary artery pressures, which can act as a surrogate for elevated left sided filling pressures can

also be used. This spectral Doppler method measures the tricuspid regurgitation (TR) jet velocity and applies the formula $4V^2 + \text{right atrial pressure}$ ($V = \text{Doppler velocity of regurgitant jet}$). Standardized measures of right atrial pressure are readily available from guideline and textbooks. While the non-invasive stress test is practical and translatable, translational gaps persists partly due to discrepancies in role of e/e' found in Burgess *et al*^[91] and Maeder *et al*^[36], identifying a suitable adjunct for pulmonary artery pressures when TR is absent, and establishing values that constitute elevated pressures across the spectrum of resting diastolic profiles, and baseline pulmonary artery pressures.

RATIONALE AND ARGUMENTS FOR FUTURE CLINICAL STUDIES OF DHF

Clinical correlation of atrial derived parameters

The current understanding of diastology does not allow us to definitively correlate symptoms to the varying changes in diastolic profiles. In addition no single parameter can be used to determine the diagnoses. In the process of grading diastolic abnormalities changes in the mitral valve velocity profiles and tissue Doppler occur as a normal part of aging. With the advent of exercise diastology and the inclusion of left atrial volume in the most recent guidelines, highlights the importance of looking for evidence that LV filling pressures are elevated in a patient with SOB. We thus feel that an important first step is to document an increase in intracardiac pressures and the subsequent steps should go on to explore the causes for this both in the LV and LA. The bases for the later is that many of the atrial derived parameters are used to define LV diastolic function, with little emphasis on how changes in LA function could alter this.

Terminology

DHF syndrome is a broad categorisation of a complex syndrome with multiple contributors where the end result is SOB and clinical impairment. Unlike SHF where the entirety of the syndrome is coupled with an impairment of LV myocardial contractility, in HFpEF it remains unclear how the interplay between degrees of LV stiffness and LA dynamics contributes to symptoms. Thus terminology in HFpEF should reflect the atrioventricular interaction in LV diastole. Lets explore several hypothetical case examples: (1) HFpEF - With predominantly impaired LV relaxation. In this scenario a patient would have clinical symptoms and signs, abnormal LV diastolic parameters, has demonstrated elevation of LVEDP (at rest or exercise), without significant LA abnormalities, and a shift of LVEDP and volume curve to the left; and (2) HFpEF - secondary to atrial dysfunction/atrial fibrillation. In this scenario the patients have similar presentation as above, however despite rate control, remains symptomatic. Restoration of sinus rhythm correlates with clinical improvement of symptoms.

Part of establishing the terminology requires an improved understanding of all aspects of LV and LA abnormalities.

Future clinical studies

The premise of any future study should be based on consolidating the diagnosis with this point in mind: "In a patient with SOB and normal LVEF the diagnosis of HFpEF can only be consolidated by reproducibly demonstrating an elevation of LVEDP or LA pressure before treatment, that this elevation is outside a physiological norm and correlates with the patients symptoms". The premise of therapeutic studies while not the aim of this paper should also focus on atrioventricular pathophysiological derangements. From this point we can explore the steps in cardiac investigations.

Screening: (1) Firstly all patients should have a screening echocardiogram; and (2) epidemiology studies are still needed to correlate the chronology of diastolic parameters with time and symptoms.

Demonstrating increased LVEDP: Firstly, we need to demonstrate an increase in LVEDP, and secondly we need to demonstrate the abnormality in the atrioventricular context. An important question then is should exercise stress testing be a routine part of DHF work-up? Due to cost, availability and the sheer volume of patients' invasive tests seem unrealistic, however non-invasive exercise echocardiography could screen patients needing an invasive test. Secondly, what parameters to use? (1) Pulmonary artery pressure elevations detected by exercise stress echocardiography can be a surrogate for LVEDP. Excluding other causes for pulmonary hypertension is important. When TR is absent patients could go onto an invasive exercise right heart study; and (2) The role of e/e' and other variations in spectral and tissue Doppler parameters

requires further attention. There is conflicting data from studies in the former and a lack of data for the latter^[36,99]. Thirdly, natriuretic peptides: Are secreted in response to atrial (atrial natriuretic peptides) or ventricular (brain natriuretic peptides) stretch. These factors have different biological properties such as chamber secreted and half-life can be exploited for diagnosis and monitoring. In clinical translation its utility with exercise stress echocardiography as a surrogate for an invasive right heart study derived LVEDP is yet to be defined^[95].

Atrial function: Is difficult to assess both at baseline and with exercise, as there are no clinically friendly tools. As many of the echocardiographic derived DHF parameters correlate with atrial mechanics, understanding how these parameters change with LA disease will better inform LV diastology. Several examples: From an invasive study in dogs undergoing exercise, it is observed that reservoir and booster functions increase but not conduit function^[96]; in 50 HFpEF patients, using late diastolic mitral annular velocity and calculated left atrial reserve index, found reduced LA function with exercise that could contribute to symptoms in addition to LV systolic and diastolic abnormalities^[97]. An improved understanding could also help inform future therapies targeting the LA.

Reliability in monitoring: Issues that need to be addressed are inter and intraobserver variability and the correlation of diastolic parameters following treatment and with changes in clinical status over time^[100].

Diastolic compensation and chronology: For patients who have abnormal baseline diastology who do not demonstrate increases in LVEDP with exercise, we will need to find satisfactory means to exclude HFpEF from the diagnosis. This will require improved understanding of diastolic compensation in the chronology of myocardial cellular function, where a different result could be elicited with different conditions.

CONCLUSION

SOB is a common symptom presentation to cardiology clinics. Clinical workup can point toward coronary artery disease, HFrEF, respiratory causes or anemia. There is also a sizable group where differentiation is required between deconditioning, obesity or HFpEF. Thus diagnosis of HFpEF has and still remains difficult where no one parameter we have is "a smoking gun". Baseline echocardiographic parameters have translated into flow diagrams published in the latest guidelines. There remain however important gaps in the understanding of this syndrome: (1) Diastolic function is complex in that it requires functional mechanics of both the atrium and ventricle, where less importance has been placed in understanding LA function; (2) exercise stress echocardiography is underutilized in the diagnostic

work-up; (3) our understanding of the baseline and subsequent parameters in its reproducibility and clinical translation requires more study; (4) the terminology defining the major contributor to HFpEF into atrial or ventricular dysfunction, should be explored; and (5) the translation of diagnostic findings into the clinical context such as relieving LVEDP, addressing myocardial stiffness with antifibrotics, correcting or augmenting atrial function and perhaps even devices to improve atrioventricular electrical or mechanical functions. To satisfactorily deliver optimal treatments more studies are needed to consolidate on our understanding and to confidently provide the diagnosis of HFpEF in a patient presenting with SOB.

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Is forced oscillation technique the next respiratory function test of choice in childhood asthma

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Abstract

Respiratory diseases, especially asthma, are common in children. While spirometry contributes to asthma diagnosis and management in older children, it has a limited role in younger children whom are often unable to perform forced expiratory manoeuvre. The development of novel diagnostic methods which require minimal effort, such as forced oscillation technique (FOT) is, therefore, a welcome and promising addition. FOT involves applying external, small amplitude oscillations to the respiratory system during tidal breathing. Therefore, it requires minimal effort and cooperation. The FOT has the potential to facilitate asthma diagnosis and management in pre-school children by facilitating the objective measurement of baseline lung function and airway reactivity in children unable to successfully perform spirometry. Traditionally the use of FOT was limited to specialised centres. However, the availability of commercial equipment resulted in its use both in research and in clinical practice. In this article, we review the available literature on the use of FOT in childhood asthma. The technical aspects of FOT are described followed by a discussion of its practical aspects in the clinical field including the measurement of baseline lung function and associated reference ranges, bronchodilator responsiveness and bronchial hyper-responsiveness. We also highlight the difficulties and limitations that might be encountered and future research directions.

Key words: Asthma; Forced oscillation technique; Impulse oscillatory; Pre-school; Children; Pulmonary function test

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Core tip: Respiratory diseases, such as asthma, are especially common in children. Although their diagnosis and management are facilitated by using spirometry in older children, the use of the latter remains limited in younger children because of their inability to perform forced expiratory manoeuvre. Therefore, the use of new methods which require minimal effort and cooperation from children, such as the forced oscillation technique (FOT) is a welcome and promising addition to identify children with underlying airway function abnormalities. In this article, we review the available literature on the use of FOT in childhood asthma.

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INTRODUCTION

Asthma is the most common chronic childhood disease worldwide^[1]. It is often difficult to diagnose in infants and young children due to lack of objective measures, such as spirometry^[2-4].

Spirometry is the gold standard method to assess lung function in older children and adults. However, obtaining acceptable and repeatable spirometry measures requires significant efforts and high level of cooperation^[5-7]. Therefore, the diagnosis and management of childhood asthma remain suboptimal, in young children and older children who cannot perform an acceptable forced expiratory manoeuvre.

One potential lung function method suitable for use in young children and older children unable to perform spirometry is the forced oscillation technique (FOT). The FOT was developed by DuBois *et al*^[8] in 1956, to measure the mechanical behaviour of the respiratory system. Over the years, the FOT has been used in research and more recently in clinical practice^[9-12]. The application of FOT has expanded to the point where commercial equipment are now widely available. Standardized approaches for the collection of FOT outcomes have been established by the European Respiratory Society (ERS) and the American Thoracic Society (ATS) in pre-school children^[9].

One significant advantage of the FOT is its application during tidal breathing and the subsequent reduction in the level of active participation and cooperation required from the individual being tested. As a result, this technique can be feasibly used in children as young as two years of age^[13]. Consequently, the FOT opens new frontiers in the application of objective measurements of respiratory function in young children and offers improvements in the diagnosis and management of asthma in young children.

In this review we have summarised the available literature on the use of FOT in childhood asthma. The

technical aspects of FOT are briefly described followed by a discussion of its practical aspects in the clinical field, including the measurement of baseline lung function and associated reference ranges, bronchodilator responsiveness and bronchial hyper-responsiveness. We also highlight the difficulties and limitations that might be encountered and future research directions.

Technical aspects of FOT

The basic principle of FOT involves the application of external signals into the respiratory system and measuring the resulting response of that system^[10]. This response is termed the respiratory system impedance (Zrs). The Zrs can be determined when flow and pressure are measured across a known frequency range at the airway opening and is represented as the resistance (Rrs) and reactance (Xrs) of the respiratory system

$$Zrs = Pao / V'ao = Rrs + j Xrs$$

where Pao is the pressure V'ao is the flow measured at the airway opening and $j = \sqrt{-1}$

Resistance (Rrs) represents the component of Zrs that is a function of both Pao and V'ao, equating to the resistive properties of the respiratory system. Reactance (Xrs) is the out-of-phase component that is a function of both Pao and volume, reflecting elastic recoil of the respiratory system. Both Rrs and Xrs are determined when oscillatory (sound waves) signals are applied at the airway opening (and hence to the whole respiratory system).

It is important to be aware that the respiratory impedance is frequency-dependent: (1) At low frequencies, (2-4 Hz) as the oscillations are transmitted more distally into the lungs, Rrs and Xrs tend to reflect the properties of the peripheral respiratory system; (2) at higher frequencies, (> 20 Hz) the Zrs reflects the resistive and inertive properties of the proximal conducting airways.

It is critical to note that Rrs and Xrs reflect mechanical properties of the entire respiratory system, including the airway, lung and chest wall^[10]. It is therefore not possible to assign specific anatomical changes (for example central airway obstruction) to changes in any one FOT outcome at a specific frequency (see below for further details).

As the airway and the lung tissue are both flow and volume-dependant, the characteristic of the oscillatory signals used is important. These signals can take any of the following common forms: (1) Single frequency; (2) impulse oscillation system^[14]; (3) pseudorandom noise (the simultaneous application of several frequency components).

The oscillation signal that is most commonly applied encompasses the medium frequency range, generally including frequencies between 2 Hz and 20 Hz. The advantage of using mid-range frequencies is that the oscillatory signals can be superimposed on the tidal breathing and therefore result in a broader application^[10]. For more details reviews on the technical aspects of FOT, readers are directed to review (oscillation mechanics of respiratory system) by Bates *et al*^[15].

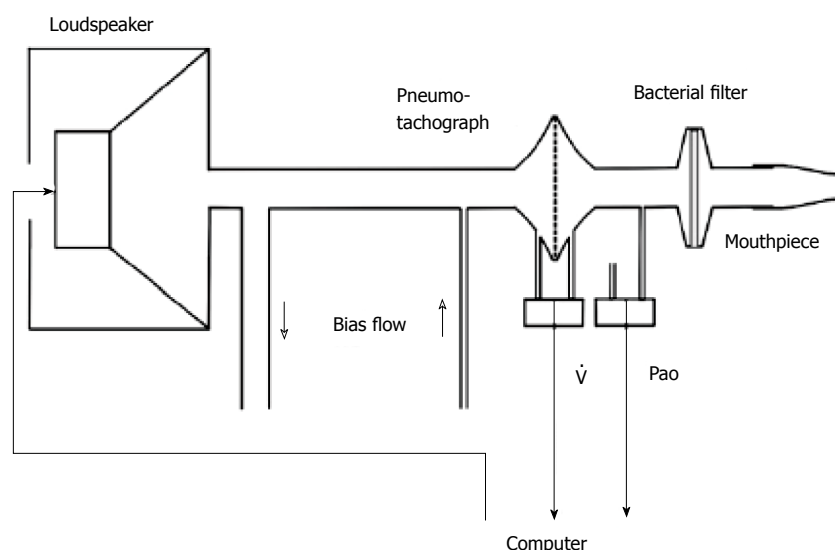


Figure 1 Typical arrangement of the forced oscillatory impedance measurement, adapted from. Pao: Input pressure at the airway opening; V'ao: Output flow.

Practical aspects of FOT

This section summarizes the available equipment, feasibility, repeatability, and finally the collection and reporting of FOT data in young children.

Equipment

The availability of FOT commercial equipment has resulted in the increased uptake of FOT in clinical practice and especially in young children. The FOT equipment includes (Figure 1): (1) A loudspeaker or similar to generate the oscillatory signals; (2) a pneumotachograph and pressure transducers to measure pressure and flow; (3) a mouth piece containing a bacterial filter to prevent cross infection between patients. A typical arrangement of the forced oscillatory impedance measurement, adapted from^[9].

Feasibility

The feasibility and success rates of FOT are, understandably, age-dependent. The success rate in children 4 years of age and older exceeds 80%^[13,16,17], while it ranges from 83% to 100% in healthy children aged 2-7 year^[13,18] and between 57% to 100% in children with asthma aged between 3 to 5 years^[13,16]. In young children with acute asthma the success rate of FOT reduced to 24% and 65% in three and eight-year-old children respectively. However, it was higher than that of spirometry in the same population^[12]. Furthermore, the feasibility of FOT measurements increases noticeably with practice in children.

The feasibility of FOT with challenge testing has been assessed in several research studies and has been shown to be feasible in young children using either inhaled adenosine monophosphate (AMP)^[19], free running^[20], methacholine^[21-23], hypertonic saline^[21], cold air^[24] or mannitol challenge^[25].

Collection of FOT data

For adequate collection of the data, the child should be

seated with their back straight and their neck either in the neutral position or slightly extended. FOT is usually performed with the mouthpiece which incorporates a bacterial filter and a nose clip-on. A staff member or a parent needs to support the child's cheeks as well as the floor of his mouth, as shown in Figure 2. An acquisition period should cover several breathing cycles, typically lasting 8-16 s. The results, computed as the mean value of the three to five acceptable measurements, also include the measurement of the coefficient of variation calculated from the standard deviation (SD) of the measurements. Acceptable measures are the one which have no artefacts such as leak, incomplete expiration, glottis closure, swallowing and the child obstructing the mouthpiece with their tongue are easily identified^[9] (Figure 2).

Repeatability

Repeatability is an important issue when considering the role of a lung function measures. The short-term repeatability of FOT in healthy children has been assessed and it is summarized in Table 1 below. The long term (two weeks) and short term repeatability were both similar^[19].

Reporting and interpreting FOT data

Commonly reported FOT outcomes include resistance (Rrs), reactance (Xrs) at different frequencies, resonance frequency (Fres), frequency dependence (Fdep), and the area under reactance curve (AX), as illustrated in Figure 3. The reported Rrs variable includes, in the same measurement, the Rrs of the airway, that of the chest wall, and that of the lung tissue. As airway Rrs dominates Rrs in the mid frequencies^[26], it can be considered a surrogate of airway resistance^[27,28]. As frequency decreases to below approximately 4 Hz Rrs will increase and include peripheral respiratory resistance and be reflective of the peripheral airways and the lung. As Xrs, on the other hand, is dominated by elastic properties of the respiratory tissue, reflecting the elastic

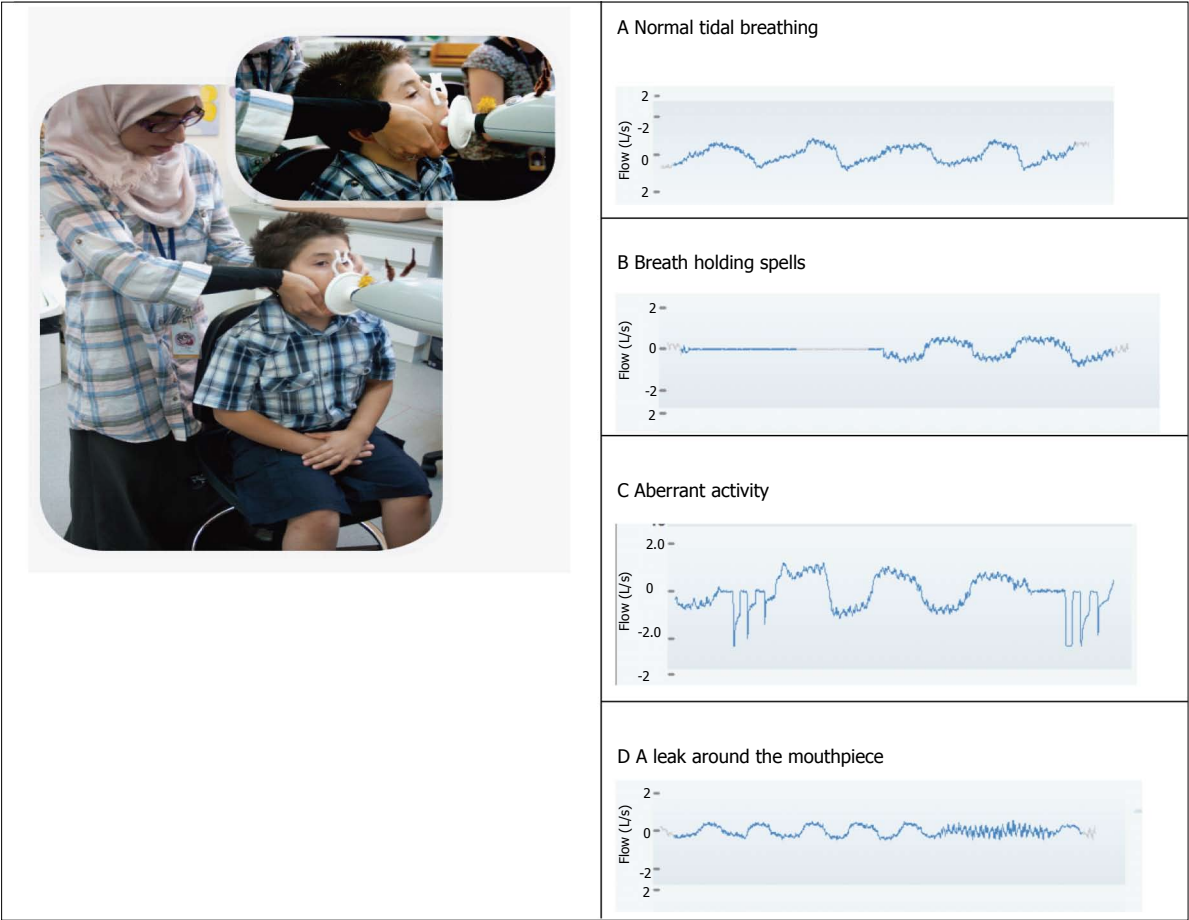


Figure 2 Demonstration of lung function measurements using FOT. On the left, a photograph of the FOT test being performed in a 5-year-old boy. The hands of the investigator support the cheeks and the floor of the mouth of the child. The nose is blocked using a nose clip. The lips are sealed around the mouthpiece. On the right, Different breathing patterns during FOT measurements are shown, as observed on the flow-time trace in L/s. A: Normal tidal breathing; B: Breath holding spells; C: An aberrant activity (e.g., coughing, swallowing, or noise); D: A leak around the mouthpiece. FOT: Forced oscillation technique.

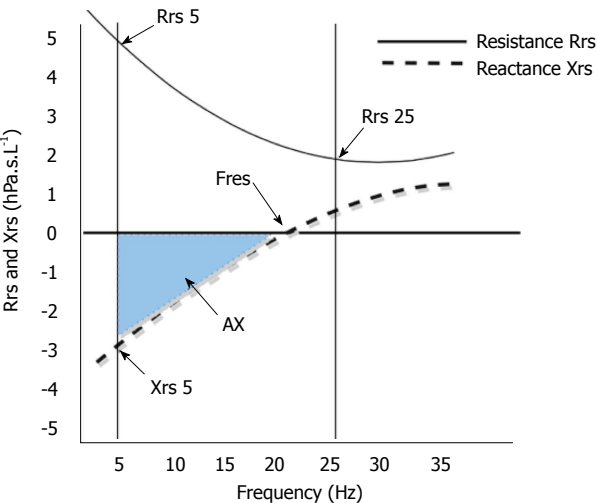


Figure 3 Changes in resistance (Rrs) and reactance (Xrs) as function of oscillation frequency.

and inertive properties of the respiratory system, it is negative at low frequencies. The point at which Xrs crosses the zero, representing the inertial properties of the larger airways^[29], is called the Fres, which is the

Table 1 Fifteen minutes' repeatability of Forced oscillation technique in healthy children

Author	Year	n	Rrs	Xrs
Hall <i>et al</i> ^[13]	2007	58; field	~2 or ~ 30%	1.2 - 1.7
Malmberg <i>et al</i> ^[18]	2002	19; placebo	1.1 or ~ 12%	1.3
Klug <i>et al</i> ^[16]	1998	120	2.6 or ~ 20%	2

Rrs: Respiratory system resistance; Xrs: Respiratory system reactance) in hPa/s per L.

frequency at which elastive and inertive properties of the lung are balanced, and which becomes positive at higher frequencies^[30]. The Fdep of that resistance is thought to reflect peripheral airway resistance^[31], as, for example, in patients with obstructive airway diseases, it is generally higher than normal subjects due to the difference in airway resistance^[30]. However, to date there are no studies directly confirming this.

The area under the reactance curve (AX) is the sum of the Xrs from a Xrs at 5 Hz until Fres (i.e., when Xrs is zero, as in Figure 3)^[29]. Studies have shown that Xrs and AX are better representative of peripheral airway obstruction than Rrs^[29] and that AX and Fres clearly

Table 2 Populations of healthy children studied using forced oscillation technique since 2005, adopted and modified from

Ref.	Year of publication	Ethnic group - Country of study	Subject number	Age in years	Height (cm)	Reported prediction equation variables
Frei <i>et al</i> ^[35]	2005	Caucasian - Canadian	222	3-10	90-155	Rrs at 5-35 Hz Xrs at 5-35 Hz Fres, AX
Dencker <i>et al</i> ^[36]	2006	Caucasian - Scandinavian	360	2-11	90-162	Rrs at 5-20 Hz Xrs at 5-20 Hz Fres
Amra <i>et al</i> ^[37]	2008	Asian - Iranian	509	5-18	127-197	Rrs at 5-25 Hz Xrs at 5-25 Hz
Nowowiejska <i>et al</i> ^[38]	2008	Caucasian - Polish	626	3-18	95-193	Rrs at 5-35 Hz Xrs at 5-35 Hz Fres
Vu <i>et al</i> ^[39]	2008	Asian - Vietnamese	175	6-11	111-154	Rrs at 8 Hz Xrs at 8 Hz
Vu <i>et al</i> ^[40]	2010	Asian - Vietnamese	95	6-11	111-134	Rrs at 8 Hz Xrs at 8 Hz
Calogero <i>et al</i> ^[41]	2010	Italian	163	2-6	101-114	Rrs at 6-10 Hz Xrs at 6-10 Hz
Park <i>et al</i> ^[42]	2011	Korean	133	3-6	95-121	Rrs at 5, 10 Xrs at 5, 10 RF, AX
Calogero <i>et al</i> ^[43]	2013	Caucasian Italian and Australian	760	2-13	90-160	Rrs at 6, 8, 10 Hz Xrs at 6, 8, 10 Hz Fres, AX
Shackleton <i>et al</i> ^[44]	2013	Mexican	584	3-5	87-119	Rrs at 6 and 8 Hz Xrs at 6 and 8 Hz
Hagiwara <i>et al</i> ^[46]	2014	Japanese	537	6-15	111-174	Rrs at 5 and 20 Hz Rrs5-20

IOS: Impulse oscillation system; Rrs: Respiratory system resistance; Xrs: Respiratory system reactance) in hPa/s per L; Fres: Resonant frequency; RF: Resonant frequency; AX: Area under the Xrs curve from 5 Hz to Fres; MF: Multi-frequency system; Fdep: Frequency dependence of Rrs between 4–24 Hz; SF: Single frequency system.

distinguish healthy children from those with small airway disease and asthma^[32].

The FOT outcomes alter with growth and therefore need to be reported as both absolute values and as a function of a predicted value. Expressing outcomes as z or SD scores is the most appropriate. Z scores allows the easy estimation of the lower limit of normal (being either -1.64 or -1.96) and avoids the diagnostic uncertainty that can arise when using percent predicted and a fixed cut off for the presence of abnormal lung function^[33,34].

Reference range of FOT measurements

Numerous studies have reported reference data in healthy children using a variety of FOT outcomes^[13,35-44]. The FOT outcomes are generally reported to change with age, height and gender. There is some variability due to the different ethnicity, gender, age, weight, height, equipment and the methodology used in those studies and it is important that users carefully review potential reference equations to match the populations, equipment and protocols used as closely as possible to their own circumstances^[37,45]. Reporting and comparing the relevant z-scores for these measurements simplifies the interpretation as the possible confounders reported earlier have already been taken into consideration when calculating these scores. Table 2 below summarises

those studies and shows those differences.

Clinical applications of FOT in children with asthma

By incorporating measurements of bronchodilator Responsiveness (BDR) and bronchial hyper-responsiveness (BHR), the utility of FOT to assist in the diagnosis of asthma in young children may be increased^[47]. The official ATS/ERS statement on pulmonary function testing in preschool children stated that FOT is a promising tool in diagnosing and following up children with asthma^[9]. Other studies also suggest that the FOT may be useful in assessing asthma control, compliance to medication, and in the follow up of these young children^[48].

Baseline FOT and the severity of asthma

In children aged 5 years and above, spirometry adequately assesses baseline lung function and the results correlate well with asthma severity^[49]. When comparing spirometry and FOT in children older than 6 years of age, those with asthma have lower baseline FOT than with spirometry when compared to healthy children^[50]. Whilst few studies have examined baseline airway obstruction in young children with asthma using FOT. Klug *et al*^[51] reported that young children with stable asthma demonstrated impaired baseline lung function when assessed using FOT. Oostveen *et al*^[17] showed that, when compared to healthy 4-year-old children,

those children with persistent wheeze had worse baseline lung function than those with transient wheeze as assessed by FOT. Children with a history of recurrent wheeze and/or asthma recruited from clinics tend to have worse lung function expressed in FOT even when asymptomatic^[18,52]. However, other studies conducted in children with history of wheeze and recruited from the community have similar FOT outcomes to healthy children^[53,54]. The ability of FOT to determine asthma severity remains therefore questionable, especially in children on asthma medication^[49]. Further studies are therefore needed to explore the relationship between baseline lung function using FOT and asthma severity in young children with asthma.

Bronchodilator response using FOT

The assessment of bronchodilator responsiveness using FOT in young children with asthma has been encouraged in clinical practice^[10]. Critical to the assessment of increased responsiveness associated with asthma is an understanding of the response of healthy children with bronchodilators. The assessment of change in FOT outcomes during the assessment of BDR has been expressed as absolute change or relative change from the baseline in the Rrs, Xrs and AX, the use of AX has been explored in a few studies and is shown to be a good outcome in assessing the BDR in children with asthma^[17,29,32].

In the most recent study which assessed BDR in children (Calogero *et al.*^[43]), cut-offs for a positive BDR in healthy Caucasian children were defined as 34% and 50% for Rrs and Xrs, respectively expressed as a relative change from the baseline lung function. In a study looking at the uses of pseudorandom FOT signal (4–48 Hz) in quantifying BDR in healthy young children, children with cystic fibrosis, neonatal chronic lung disease and children with asthma and/or current wheeze, Thamrin *et al.*^[53] recommended a positive BDR response of 40% and 65% for Rrs and Xrs, respectively expressed as a relative change from the baseline lung function^[53]. Another study, conducted by Oostveen *et al.*^[17] in Belgium on 4 years old healthy and wheezy children ($n = 325$) using FOT, recommended a positive BDR at Rrs of 43% expressed as absolute changes. Cut-off values for BDR in previous studies are summarized and listed in Table 3. In general, > 30% decrease in Rrs after bronchodilator is suggestive of asthma.

The previous studies in healthy and wheezy young children have not conducted a ROC analysis to formally establish the sensitivity and specificity of a certain BDR cut off to be assessed. Limitations to the wider use of FOT in assessing BDR include the variability of medications, their timing and dosage between the different studies (Table 3).

Bronchial hyper-responsiveness using FOT

Bronchial hyper-responsiveness, usually assessed using spirometry, is the gold standard for confirming a

diagnosis of asthma in older children and adults^[58,59]. However, false negative or false positive results can occur in young children who cannot perform an acceptable manoeuvre. BHR has been assessed in young children using other methods including FOT, interrupter technique, whole-body plethysmography and transcutaneous oxygen measurement SPO₂^[5,47,60-63].

Bronchial hyper-responsiveness studies comparing FOT with different lung function measures have reported FOT to be as effective as spirometry^[63-69] and as sensitive as body plethysmography^[70] and transcutaneous PO₂^[50] in older children with asthma. BHR has been assessed using FOT in children younger than 7 years of age with a range of challenge tests including adenosine monophosphate (AMP)^[19], cold air^[24], normal saline^[21] methacholine^[22,62,63,66,68,71], mannitol^[25] and exercise^[20,72] demonstrating that FOT can be reliably used in young children for challenge testing.

FOT characterization of BHR has been shown to be correlated to asthma severity^[73] and to be sensitive to the response to immune therapy^[74]. Another study has shown that cough variant asthma showed less BHR in comparison to classical asthma children^[75].

Despite the above studies have clearly demonstrated the use of FOT to assess BHR in children, there are few studies demonstrating the best cut off value of BHR between healthy and children with asthma using FOT. Further work is needed before establishing FOT use as clinical tool to assess BHR in children.

Limitation of FOT in children and future direction

Although forced oscillation technique can contribute to the diagnosis and management of childhood asthma, it has some limitations. Although, unlike spirometry, it does not require forced expiratory manoeuvres, it still require some cooperation by children to achieve successful repeatable measures. It is therefore both age and cooperation dependant. The practical advantage of the availability and affordability of different FOT commercial equipment, the Rrs and Xrs output are not always measured at the same frequencies by all these devices. This makes the comparison between different studies challenging. The standardization of the available commercial equipment is therefore still needed and FOT guidelines are currently being reviewed by the ATS and ERS.

As the available FOT reference equations have been constructed in specific populations, these findings cannot be generalised to other ethnic groups. Further studies are therefore still required to establish FOT reference values in other populations or ethnic groups. The assessment of BDR using FOT is not widely implemented because of differences amongst the relevant studies, including differences in the medications used, as well as their timing and dosage. Further studies are therefore required to establish an international standard protocol for this assessment as currently BDR still has not been studied in relation to recent symptoms in young children with wheeze, developing such studies

Table 3 Reported bronchodilator responsiveness in young healthy and wheezy children using forced oscillation technique since 2005

Ref.	Ethnic group age, yr	n	Variable	Dose	Waiting time (n)	Absolute BDR	Relative BDR, (%)
Thamrin <i>et al</i> ^[53] , 2007	Healthy Caucasian (3-7)	78	Rrs 6,8,10 Xrs 6,8,10	Salbutamol 600 mg	15	Rrs 6 -35.0, -3.1 Rrs 8 -35.0, -4.4 Rrs 10 -32.3, -3.7 Xrs 6 -0.27, 1.66 Xrs 8 -0.04, 1.82 Xrs 10 -0.00, 2.03	Rrs 6: 42% Rrs 8: 37% Rrs 10: 39% Xrs 6: 61% Xrs 8: 67% Xrs 10: 63%
Thamrin <i>et al</i> ^[53] , 2007	Asthmatics Caucasian (3-7)	57	Rrs 6, 8, 10 Xrs 6, 8, 10	Salbutamol 600 µg	15	Rrs 6 -37.0, 8.8 Rrs 8 -33.1, 9.7 Rrs 10 -33.4, 4.5 Xrs 6 -0.21, 2.30 Xrs 8 -0.13, 2.47 Xrs 10 -0.12, 2.22	Rrs 6: 42% Rrs 8: 37% Rrs 10: 39% Xrs 6: 61% Xrs 8: 67% Xrs 10: 63%
Lan Vu <i>et al</i> ^[39] , 2008	Healthy Vietnamese (6-11)	175	Rrs 8 Hz Xrs 8 Hz	Salbutamol 200 µg	5	Rrs 8 -11.8,13.4 Xrs 8 4.09, - 5.78	Rrs 8: 38% Xrs 8: 16%
Lan Vu <i>et al</i> ^[40] , 2010	Asthmatic Vietnamese (6-10)	103	Rrs 8 Hz Xrs 8 Hz	Salbutamol 200 µg	5	--	Rrs 8: 13% Xrs: 32%
Oostveen <i>et al</i> ^[55] , 2010	Asthmatic Belgian -4	313	Rrs 4	Salbutamol 200 µg	15	--	Rrs 4: 22% AX: 15.77%
Calogero <i>et al</i> ^[41] , 2010	Healthy Italian (3-6)	163	Rrs 8 Xrs8	Salbutamol 200 µg	15	--	Rrs 8: 35% Xrs 8: 34%-61%
LEE <i>et al</i> ^[57] , 2012	Healthy Korean	161	Rrs 5, 10, 15, 20, 25, 35 Xrs 5, 10, 15, 20, 25, 35	Salbutamol 200 µg	15	Rrs 5 -0.127 Rrs 10 -0.098 Rrs 15 -0.073 Rrs 20 -0.056 Rrs 25 -0.056 Rrs 35 -0.057 Xrs 5 0.062 Xrs 10 0.057 Xrs 15 0.059 Xrs 20 0.044 Xrs 25 0.033 Xrs 35 0.054 Fres -2.167	Rrs 5: 11.8 Rrs 10: 10.8 Rrs 15: 8.7 Rrs 20: 6.972 Rrs 25: 7.029 Rrs 35: 6.095 Xrs 5: 13.474 Xrs 10: 25.946 Fres: 10.457
Calogero <i>et al</i> ^[43] , 2013	Healthy Australian and Italian (2-16)	502	Rrs 6, 8, 10 Xrs 6, 8, 10 Fres, AX	Salbutamol 200 µg	15	Rrs 6 -2.9 Rrs 8 -2.74 Rrs 10 -2.39 Xrs 6 -1.80 Xrs 8 -1.93 Xrs 10 -1.90 AX -33 Fres -12	Rrs 6: 34 Rrs 8: 32 Rrs 10: 31 Xrs 6: 50 Xrs 8: 65 Xrs 10: 74 AX: 81 Fres: 47

BDR: Bronchodilator responsiveness; Rrs: Respiratory system resistance; Xrs: Respiratory system reactance in hPa/s per L; Fres: Resonant frequency; AX: Area under the Xrs curve from 5 Hz to Fres; MF: Multi-frequency system; Fdep: Frequency dependence of Rrs between 4-24 Hz.

would help not only in the follow up of children with wheeze, but also to ascertain the level of control of asthma symptoms and the compliance to medication in children with asthma.

Further research is also required to assess BHR, particularly in young children, in addition to standardise the use of FOT in young children for BHR assessment.

Recently there are new studies that have reported Rrs or Xrs from either expiration or inspiration (or both), including flow limitation within a breath. Those studies suggest that this approach is more sensitive than standard reporting of FOT. However, significant work is required prior to the introduction of these outcomes into clinical practice and this remains an area for the future studies^[76,77].

In conclusion, with the relatively high prevalence of childhood asthma, FOT has been proven to be a useful

tool to aid in its diagnosis and management especially in children unable to perform spirometry. As the recent availability of commercial equipment has increased its use both in research and in clinical practice, clinicians have to understand the emerging role of FOT in clinical practice and how to interpret its results in order to optimise clinical management of children with asthma.

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Basic Study

Quantitative comparison of cranial approaches in the anatomy laboratory: A neuronavigation based research method

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Data sharing statement: ApproachViewer guide 1.0 is available from the corresponding author at francesco.doglietto@unibs.it.

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Abstract

AIM

To describe the development and validation of a novel neuronavigation-based method, which allows the quan-

tification of the anatomical features that define an approach, as well as real-time visualization of the surgical pyramid.

METHODS

The method was initially developed with commercially-available hardware for coordinate collection (a digitizer and a frameless navigation system) and software for volume rendering; dedicated neuronavigation software (ApproachViewer, part of GTx-UHN) was then developed. The accuracy of measurements and the possibility of volumetric rendering of surgical approaches simulated in a phantom were compared among three different methods and commercially-available radiological software. In the anatomy laboratory, ApproachViewer was applied to the comparative quantitative analysis of multiple neurosurgical approaches and was used by many surgeons who were untrained for the research method.

RESULTS

The accuracy of ApproachViewer is comparable to commercially-available radiological software. In the anatomy laboratory, the method appears versatile. The system can be easily used after brief training. ApproachViewer allows for real-time evaluation and comparison of surgical approaches, as well as post-dissection analyses of collected data. The accuracy of the method depends on the navigation registration: with a 1-2 mm registration error, it is adequate for evaluation and comparison of most neurosurgical approaches.

CONCLUSION

This new research method and software allows semi-automated visualization, quantification, and comparison of neurosurgical approaches in the anatomy laboratory.

Key words: Anatomical study; Comparison; Neurosurgical approach; Quantification; Research method; 3D rendering

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Core tip: Herein, the authors describe the development and validation of a novel research method that allows quantification of the essential anatomical features of a neurosurgical approach and real-time rendering of its surgical volume. The measurements of ApproachViewer in a phantom with simulated approaches were noted to be largely homogeneous and comparable with those of other research methods. The authors further demonstrated the actual application of ApproachViewer in anatomical dissections to elaborate the advantages of real time 3D rendering and quantification. ApproachViewer provides a good alternative solution for fast 3D rendering and post-dissection analyses in a preclinical setting.

Doglietto F, Qiu J, Ravichandiran M, Radovanovic I, Belotti F, Agur A, Zadeh G, Fontanella MM, Kucharczyk W, Gentili F. Quantitative comparison of cranial approaches in the anatomy laboratory: A neuronavigation based research method. *World J*

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INTRODUCTION

Anatomical research is an essential phase of modern surgical innovation and evaluation^[1], especially in an era of technological advancements, leading to the continuous improvement of different surgical approaches and techniques^[2,3]. In the last two decades, different methods have been described for the quantification and comparison of neurosurgical approaches in the anatomy laboratory^[4]. A common limitation of currently available research methods is the lack of visualization of the surgical pyramid that anatomically defines a neurosurgical approach (Figure 1)^[4,5].

Herein, we present a novel neuronavigation based method that allows real-time visualization of the surgical pyramid, as well as quantification of its features and various post-dissection analyses. The development and validation process is described, together with some examples of the application of the method to evaluation and comparison of neurosurgical approaches in the anatomy laboratory.

MATERIALS AND METHODS

Four image-based systems were used to quantify and visualize the surgical pyramid^[4,5] (Figure 1) in five simulated approaches (Figure 2). Every simulated approach was quantified 10 times with each research method. Data were then analyzed for intra- and inter-method variability (Figure 3). Two of the methods, based on neuronavigation systems, were also used to quantify surgical approaches in the anatomy laboratory.

Computed tomography scan

Computed tomography (CT) scans of phantom and anatomical specimens were performed using a 1 × 1 frame with contiguous slices, both at 1 and 3 mm (Somatom Definition Flash®, Siemens, Forchheim, Germany). CT was performed at a gantry of 0°, with a scan window diameter of 225 mm and a pixel size of more than 0.44 × 0.44. Images were recorded on a CD in DICOM format.

Quantification methods

The four research methods that were used to quantify and visualize the simulated surgical approaches are: (1) Radiological software (eFilm™); (2) digitizer and 3D rendering software (Autodesk Maya®); (3) navigation system and Autodesk Maya®; and (4) navigation hardware and a dedicated, new navigation software (ApproachViewer, part of GTx-UHN - Guided-Therapeutics software developed at University Health Network - Toronto, Canada).

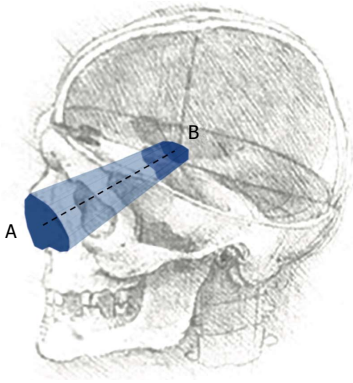


Figure 1 Schematic description of a surgical approach, as a “truncated pyramid”^[4,5]: (1) Superficial surface (A), more commonly defined “surgical window” or “access area”; it represents the area through which instruments are introduced to work at the level of the deep area; (2) deep surface (B), usually defined “area of exposure”, as it is the surface exposed by the approach; and (3) height of the truncated pyramid (dotted line). Volume and trajectory of this pyramid complete the essential anatomical definition of a neurosurgical approach. Background head image is from: Studies of human skull, Leonardo Da Vinci, supplied by Royal Collection Trust ©Her Majesty Queen Elizabeth II 2016.



Figure 2 A phantom was used to evaluate the accuracy of the methods used to quantify the surgical pyramid. Three funnels were positioned inside a radiotherapy mesh plastic mask to simulate a total of five different surgical approaches: Small funnel, simulating a narrow and short approach; medium funnel (asterisk), simulating two different approaches, with the same superficial area and different deep areas, at distinctive distances from the surface; large funnel, simulating two approaches, with the same superficial area and different deep areas, at the same distance. Round, one-millimeter metallic markers were positioned over the mask as reference points for neuronavigation registration (black arrow). A reference frame (white arrow) was attached to allow the use of navigation systems (see text for further details).

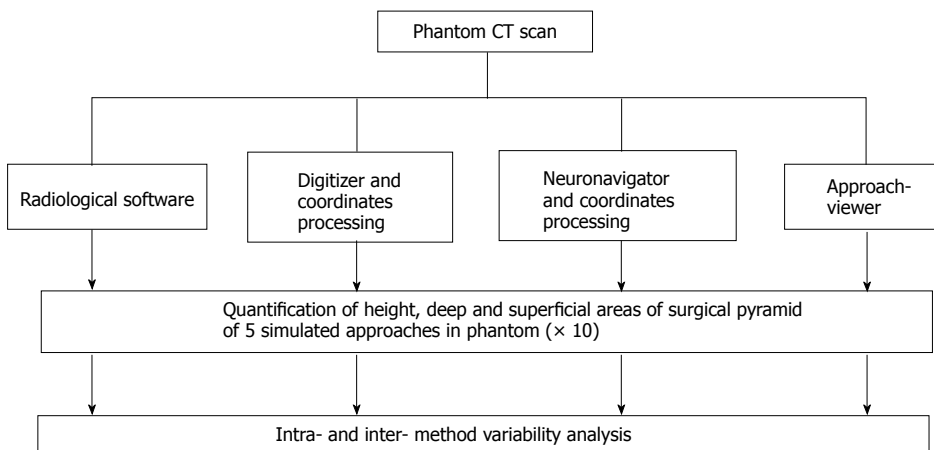


Figure 3 Study design. Each simulated approach was quantified 10 times with each of the research methods (see text for further details). CT: Computed tomography.

Radiological quantification: Commercially available radiological software that allows visualization in 2D and 3D of DICOM images was used (eFilm™, Merge Healthcare Incorporated)^[6] to visualize and quantify the simulated approaches. The superficial and deep surfaces were calculated by dividing them in eight triangles; each triangular area was measured using Heron's formula (Figure 1 of online additional material). The height of the surgical pyramid was measured with the tool for linear distances (Figure 2 of online additional material)^[6]. Three-D rendering of the approaches was performed, when feasible, using the volume rendering tools^[6] (Figure 3A of online additional material).

Digitizer and Autodesk Maya®: Microscribe™ 3DX Digitizer (Immersion Corporation, San Jose, CA, United States) consists of a base and digitizing arm with five degrees of freedom that terminates in a fine tip stylus (Figure 4 of online additional material). When

the stylus tip is placed on a surface and a button is pressed, the Cartesian coordinates (x, y, z) of the point are recorded and stored in a computer as a text file. The digitizer mean accuracy is 0.23 mm^[7]–0.5 mm^[8].

Commercially-available software for image-based modelling and rendering (Autodesk Maya®) was used to build a 3D model of surgical approaches using collected coordinates (see Figure 3B of online additional material). MEL Script and Python programming languages were used to create a plug-in to import the coordinates into Autodesk Maya®. The resulting model could be manipulated in 3D and allowed visualization from any view. Using MEL, a script was created to measure: (1) Distance: The height of the truncated pyramid was calculated as the distance between the center points of the superficial and deep areas. The center points were determined by the centre of mass equation, using the 8 coordinates collected along the border of both superficial and deep surfaces; and (2) Area: The area

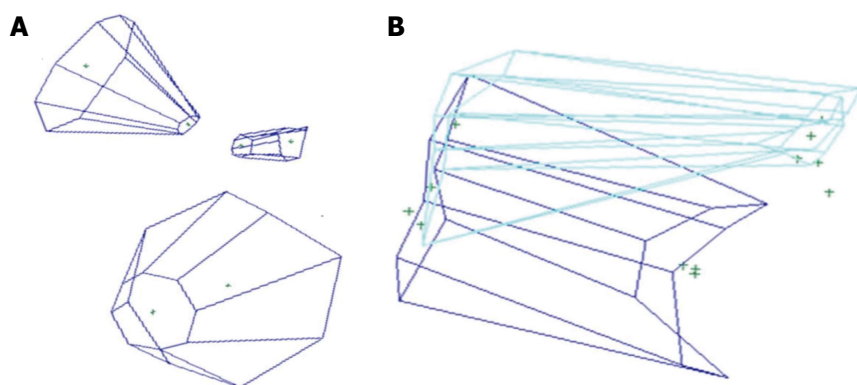


Figure 4 Volume rendering with Maya®. A: Visual rendering of three different funnels with the quantification method based on the commercially-available digitizer or navigation system and 3-D rendering software (Maya®); B: 3D rendering with the Storz navigation system for coordinate collection and Maya® software for 3D rendering of endonasal (light blue) and transoral (dark blue) approaches to the anterior craniovertebral junction.

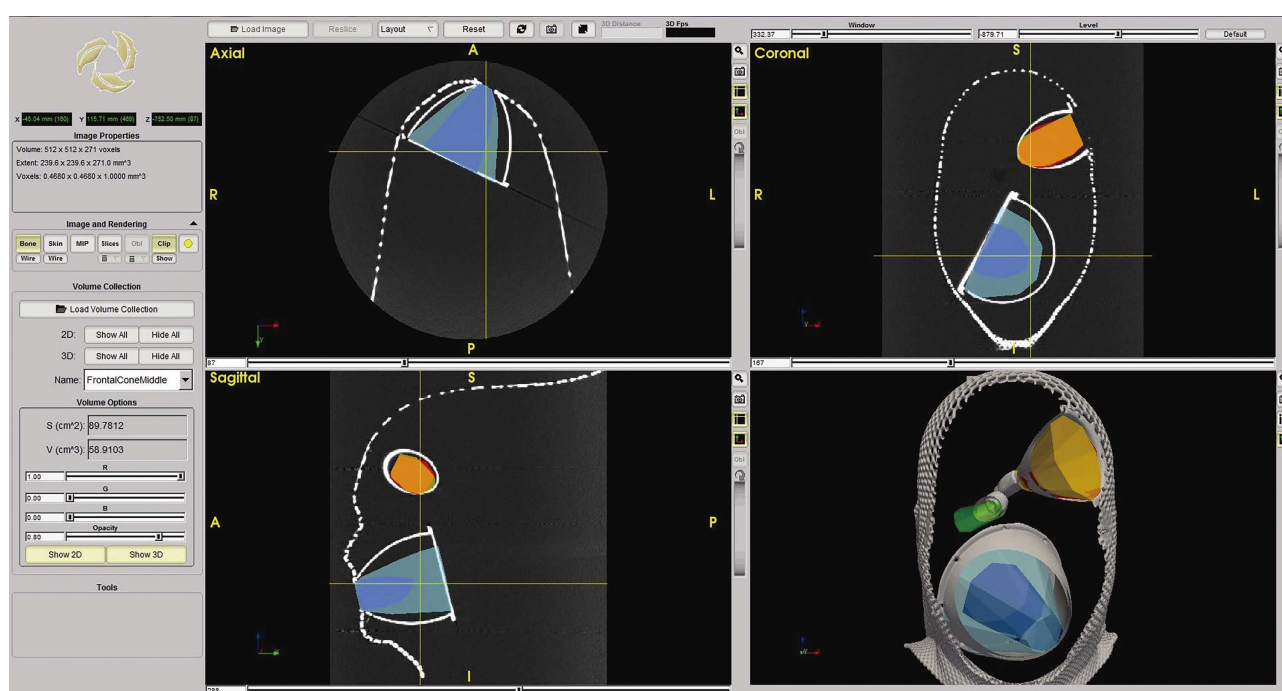


Figure 5 Rendering of the three funnels with ApproachViewer, which shows the five different approaches (in different colors) in the three axes and in 3D. The quantified volume of one of the simulated approaches is shown on the screen (see online additional material - ApproachViewer guide 1.0)

was calculated using the triangulation method. A vector was drawn to each of the 8 registered points from the center of mass equation. Two adjacent points along with the center formed a triangle, for a total of 8 triangles. Each triangular area was measured using Heron's formula.

Neuronavigation system and Autodesk Maya®: In the first phase of this study, we used a prototype created by Storz Company for ENT surgery (Karl Storz® ENT navigation system - Tuttlingen, Germany). The registration was based on four-point collection and neuronavigation was allowed when the mean registration error was less than 2 mm. This neuronavigation system provided the on-screen coordinates of a given point in millimeter values, including decimals (Figures 5 and 6 of online

additional material). A screenshot of each point of interest was recorded using a commercially-available frame grabber (VGA2USB LR frame grabber, Epiphan, Ottawa, Canada) (see Figure 6 of online additional material). The coordinates were recorded on an Excel sheet, and then imported and elaborated in Autodesk Maya®, as described above.

ApproachViewer: Dedicated software, called ApproachViewer, part of GTx-UHN (Guided-Therapeutics software developed at University Health Network - Toronto, Canada), was developed to visualize the surgical pyramid inside the head in which the approach was being performed.

The used navigation hardware by NDI (Northern Digital Imaging®, Waterloo, Ontario, Canada) included:

Table 1 Results of the phantom quantification with 4 different research methods (see text for further details)

		e-Film™		Microscribe 3DX Digitizer®		Storz® navigator + Maya® reconstructions		ApproachViewer - GTx-UHN	
		mean	SD	mean	SD	mean	SD	Mean	SD
Height (mm)	Large MS	67	0.67	67.8	0.79	66.36	0.29	69.19	0.26
	Medium	48.3	0.48	52.5	0.23	47.51	0.31	48.7	0.55
	Small	38.3	0.67	40.46	1.11	36.98	0.56	38.9	1.03
Area (mm ²)	Large - deep	5310.19	43.50	4877.78	52.82	5461.03	35.56	6003.2	49.42
	Large - deep mid	1805.37	19.64	1548.06	15.45	1836.83	32.66	2073.4	40.85
	Large - superficial	441.87	21.22	416.81	12.11	409.05	12.62	533.3	16.44
	Medium - deep	NA	NA	128.24	5.45	136.89	4.73	201.4	7.41
	Medium - middle	NA	NA	498.69	14.01	NA	NA	470.4	21.80
	Medium - superficial	2425.57	46.34	2375.56	35.35	2555	20.86	2955.7	58.89
	Small - deep	82.49	6.37	108.34	4.92	90	4.24	125.4	6.88
	Small - superficial	150.84	12.70	161.43	5.54	168.3	11.36	256.1	11.40

Area values are expressed in mm²; height values are in mm. SD: Standard deviation; Mid: Area included in the deep surface (mid-area); NA: Not available.

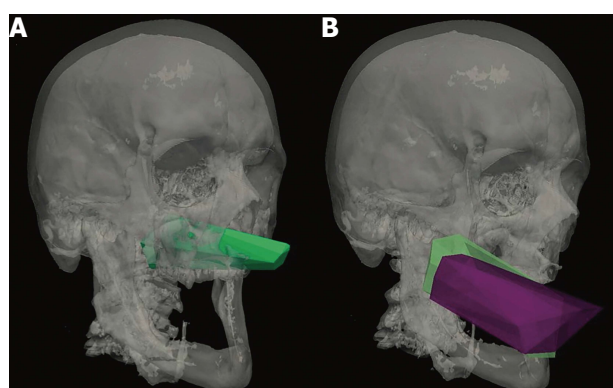


Figure 6 Visual rendering of anterior approaches to the odontoid with ApproachViewer. A: Endonasal endoscopic approach to the anterior craniovertebral junction (CVJ); B: Transoral without (purple) and after soft palate split (green) approaches to the CVJ.

(1) A passive rigid body (Rigid body 2®); (2) a passive probe with 4 markers; and (3) Polaris Vicra Optical Tracking System.

Circumferential points of the superficial and deep surfaces of each funnel were collected using the pointer tool. The surface points were then projected to the best plane fit using linear least squares. Delaunay triangulation was used to generate triangles on the plane and Heron's formula applied. The height of each funnel was measured as the distance between the center of the superficial and deep surfaces.

Data collection and statistical analysis

Each simulated approach was quantified 10 times with each research method. Mean values and standard deviation of each value were calculated. Data were collected in Excel (Microsoft®, United States) and analyzed with OriginPro® (OriginLab®, Northampton, MA, United States). Standard deviations of the different methods were compared calculating the coefficients of variation (CVs) for each, in order to test their precision^[9]. In addition, Brown-Forsythe tests for homogeneity of variances (HOV) were performed by comparing ApproachViewer to the

other quantification methods. The level of significance for Brown-Forsythe test was set at 0.05.

The statistical methods of this study were reviewed by Elena Raffetti from Epidemiology and Public Health Intervention Research group (EPHIR), Department of Public Health Sciences, Karolinska Institutet.

Anatomical dissections

Anatomical dissections and data collection were performed at the University of Toronto Surgical Skills Centre at Mount Sinai Hospital. REB approval was obtained (UHN REB number: 09-0130-T) (Figure 6 of online additional material).

Dissections were performed under 3 to 40 microscopic magnifications (Carl Zeiss® Surgical Microscope) and with 0° and 30°, 4 mm endoscopes (Karl Storz® - Tuttlingen, Germany). Various microsurgical and endoscopic approaches to the skull base and to certain intracranial areas were performed. In the first phase of this study, they were quantified with a commercially-available neuronavigation system connected to a frame grabber and coordinate elaboration with Autodesk Maya®, as described above. In the second phase of the study, ApproachViewer was always used.

RESULTS

Comparative analysis

Table 1 summarizes the results of the quantification of height, deep, and superficial areas of the phantom funnels with all 4 methods. CVs were less than one in all cases, indicating that all methods have low variance distributions.

The Brown-Forsythe tests showed that variances of ApproachViewer quantifications were homogeneous with those of the other methods, with two exceptions. Homogeneity of variances (HOV) was not verified comparing ApproachViewer to the digitizer for either medium or large funnels heights. On the contrary, HOV was statistically valid comparing ApproachViewer to e-Film and Storz navigation quantifications ($P < 0.05$).

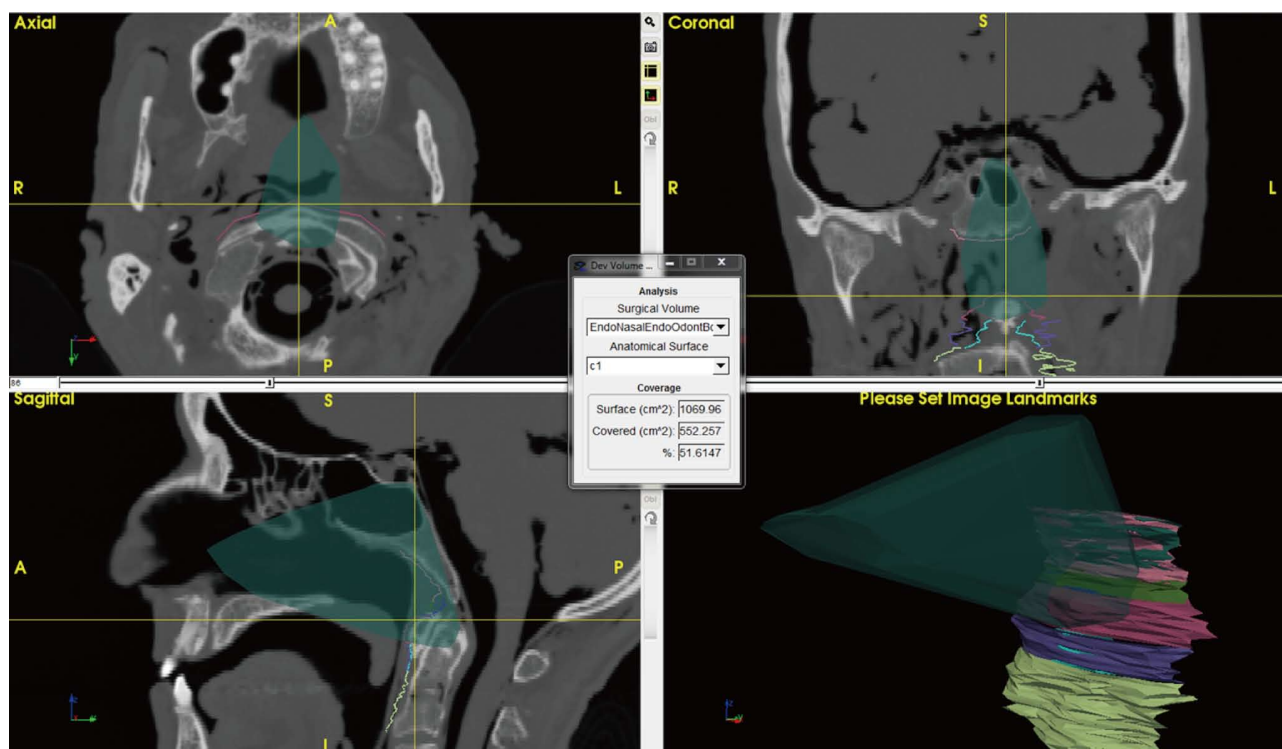


Figure 7 Detail of ApproachViewer screen during post-dissection analyses. An endonasal endoscopic approach to the odontoid was performed in the anatomy laboratory and is retrieved and visualized in the three axes and as a volume. Different areas of interest have been contoured at the level of the anterior craniovertebral junction. The box at the center shows the absolute and percentage value of contoured C1 exposed by the approach (see online additional material - ApproachViewer guide 1.0 for further details).

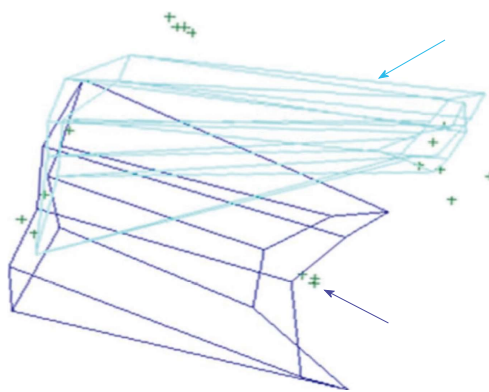


Figure 8 Three-D rendering of endonasal endoscopic (light blue) and transoral with soft palate split (purple) approaches to the anterior craniovertebral junction

3D visualization

Figures 4A and 5 show volume rendering of the simulated approaches, obtained with newly developed system (*i.e.*, navigation system and Maya® software; ApproachViewer - GTx-UHN). The results are visually comparable to the those obtained with commercially-available radiological software (eFilm™) (Figure 3 of online additional material).

Anatomical dissections

Figures 4B and 6 show 3D rendering of surgical app-

roaches that were quantified with both navigation-based research methods (*i.e.*, navigation system and Maya® software; ApproachViewer - GTx-UHN) in the anatomy laboratory (see also Figures 7 and 8 of online additional material for examples of rendering with the navigation system and Maya® software).

The mean time to obtain the 3D rendering of an approach with the navigation system and Maya® software was approximately 2-4 min for coordinate collection (8 points for each of the surfaces were collected; 2 people were needed), 3-8 min for coordinates registration in an Excel file (whether performed by one or two people), and 5 min for rendering with Maya®. The mean time to obtain the 3D rendering of an approach with ApproachViewer was 2 min, *i.e.*, the time needed to collect multiple points both at the deep and superficial surface. Rendering was therefore immediate with ApproachViewer, while it was possible only after the laboratory session with the previously developed system.

Additional features were developed with ApproachViewer, which was the only system that allowed visualization of approaches taking place inside the head in which they were performed. Areas of interest were contoured: ApproachViewer automatically calculated the absolute and percentage value of the area included in a specific approach (Figure 7). This feature was used in the post-dissection phase of anatomical studies (ApproachViewer guide 1.0 for further details, available from the corresponding author).

DISCUSSION

Comparative analysis

The comparative analyses documented a satisfactory, limited variation in the calculated data for each of the research methods, according to their CVs. Variation was more significant when different research methods were compared. We believe that this variability is due to different reasons, as recently reported by Ammirati's group^[9], possibly including different registration errors, varying methods to calculate the areas, and differing methods to collect the coordinates.

A digitizer is possibly the instrument for coordinate collection with the lowest error (0.23 mm^[7] - 0.5 mm^[8]). Its role in the analysis of surgical approaches is, however, limited by the physical constraints caused by its dimensions and desktop-based nature. The only statistically significant differences between the different methods were most probably due to the difference in coordinate collection, due to the physical constraints caused by the digitizer arm. Frameless navigation systems might have relatively higher errors, but have the advantage of high flexibility for coordinate collection, and have therefore been used extensively in recent anatomical studies^[4]. The registration error of neuronavigation can be decreased using well recognizable registration points, such as screws that can be positioned around the area of interest^[9].

3D visualization and quantification

The research method based on the use of a commercially-available navigation system and Maya® 3D rendering software provided 3D visualization and quantification of the truncated surgical pyramid that defines a surgical approach. A visual comparison of different approaches was therefore possible (Figure 4B), but to provide the correct orientation in space of each surgical pyramid a common registration had to be provided (*i.e.*, three fixed points were registered during each session). This system, which was developed in 2009, provided for the first time the visualization in 3D of the surgical approach. The method is similar to others that have been recently published^[4]. An evident practical limit of the method was the lengthy data elaboration process, which included manually copying the coordinates from each screenshot in an Excel sheet and manually superimposing the registration points in the 3D rendering of the pyramid. These issues may have been partially resolved, but the main limit that stopped us from further developing this research method was the impossibility of automatically visualizing the head in which the coordinates had been collected. It would have been possible to manually superimpose surgical volumes and CT images, but this would have introduced another possible cause of error and bias.

ApproachViewer in the anatomy laboratory

ApproachViewer was therefore developed as it allows visualization of the surgical volume inside the specimen

in which the approach has been performed.

A new feature was then developed to further advance the objective evaluation of neurosurgical approaches. Once dissection sessions are over, areas of interest can be contoured. The software then allows the automatic quantification of absolute and percentage values of the area of interest that is included in each surgical approach (Figure 7 and ApproachViewer guide 1.0). We believe that this brings to reality a research method that was foreseen by Andaluz *et al.*^[5] more than 10 years ago: "We envision the ideal comparison between approaches as a volumetric analysis of truncated pyramids with polyhedral bases, with a special interest in the top of the pyramid. The incremental presence of computer-assisted devices in cranial base laboratories may some day bring this near-science-fiction concept into real numbers".

When compared to other research methods based on navigation systems, ApproachViewer provides quantifications with the same precision, direct visualization of the surgical pyramids, and the possibility of using tools to measure areas of sections of the pyramids and to measure the distances between them to obtain heights (such as the middle surface of the medium funnel in the present study, which could not always be obtained with other methods). Compared to how we started, when we had to manually transcribe each coordinate into a separate database, which would then be elaborated, ApproachViewer is incredibly more user-friendly and faster, with automatic recording and calculations of pointer movements and positions, and thus automatic determination of pyramid dimensions and volumes. Use of the system should be intuitive to neurosurgeons who use any neuronavigation system in their surgical practice, as the same instruments, reflective spheres, and stereoscopic camera are used. Moreover, the system was used extensively by surgeons and medical students at the University of Brescia: Its use appears to be intuitive and easy to use (see ApproachViewer guide 1.0), although further studies are necessary to confirm these preliminary data.

The group led by Ammirati has recently described a method that allows visualization and quantification of the volume of a surgical approach^[4,10]. The method is based on filling the surgical cavity with fat and CT scanning after dissection; the volume is then quantified with neuronavigation software. The main limit of the method is the need for post-dissection CT after each approach is performed^[4], while ApproachViewer allows the collection of multiple data in a dissecting session without the need for multiple CT scans. On the other hand, it quantifies the surgical pyramid defined by a deep area and superficial one, which need to be defined before the dissections are performed.

The potential applications of ApproachViewer are multiple: (1) It provides a user-friendly method to quantify and therefore compare different surgical approaches; (2) as the working volume of the approach is immediately visualized on a screen, it can provide

immediate feedback to a surgeon in training; and (3) the quantification of the working space has important implications for the development of angled instruments, especially for endoscopic skull base surgery.

In the past few years ApproachViewer has been systematically applied to the analysis of multiple skull base approaches: Some results have already been published^[11-14]. All these potential applications require specific studies, possibly by multiple centers.

We have developed and validated a new, navigation-based tool for anatomical research in neurosurgery. ApproachViewer (part of part of GTX-UHN - Guided-Therapeutics software developed at University Health Network - Toronto, Canada) provides the possibility of multiple data collection during dissection sessions, as well as various post-dissections analyses, including 3D rendering of the surgical volume, quantification of the anatomical features that define a surgical approach, and of the area of interest exposed.

COMMENTS

Background

Anatomical studies are an essential part of the preclinical phase of surgical research. They can provide objective, quantitative, comparative data that can be used to evaluate novel surgical approaches and techniques.

Research frontiers

Different research methods have been described to quantify and compare different surgical approaches and most of them do not provide visual rendering and quantification of the surgical pyramid, which is the working volume that defines a neurosurgical approach.

Innovations and breakthroughs

This study describes the development and validation of a novel neuronavigation-based method, which allows the quantification of the anatomical features that define an approach, as well as real-time visualization of its surgical pyramid.

Applications

ApproachViewer has already been extensively used in the anatomy laboratory to quantify and compare different neurosurgical approaches. It provides a real-time visualization and quantification of the surgical pyramid. Possible applications include not only the preclinical quantification of surgical approaches, but also: (1) Immediate feedback to surgeons in training in the anatomy laboratory during dissections; (2) Detailed analysis of the surgical volume for the development of new surgical instruments; and (3) Quantification of the working volume in clinical scenarios.

Terminology

Neuronavigation: Computer-assisted technologies used to guide or "navigate" within the intracranial space.

Peer-review

The exposition is well conducted and the topic is very interesting.

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E- Editor: Lu YJ



Laparoscopic-extracorporeal surgery performed with a fixation device for adnexal masses complicating pregnancy: Report of two cases

Hanako Kasahara, Iwaho Kikuchi, Aya Otsuka, Yoko Tsuzuki, Michio Nojima, Koyo Yoshida

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Author contributions: Kasahara H and Kikuchi I contributed equally to this work; Kasahara H, Kikuchi I, Otsuka A and Tsuzuki Y performed the research; Kikuchi I designed the research; Nojima M and Yoshida K conducted the research; Kasahara H wrote the paper.

Informed consent statement: Because the patients have moved, we could not contact them. Before the operation, we have got comprehensive agreement, so the IRB approved.

Conflict-of-interest statement: None.

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Abstract

The potential complications associated with an adnexal mass discovered during early pregnancy call for surgical treatment. Ideally, surgery is performed after gestational week 12, but uterine expansion after the first trimester makes surgery difficult. We report two pregnancies complicated by adnexal masses for which we used an organ fixation device for safe performance of single-site umbilical laparoscopic surgery. Pelvic magnetic resonance imaging depicted a dichorionic, diamniotic twin pregnancy and 60-mm right adnexal mass in the first patient and bilateral adnexae in the second. All three masses were suspected mature cystic teratomas. Both patients underwent laparoscopic surgery during gestational week 14. With use of an organ fixation device, traction was applied until the mass reached the umbilicus; tumor resection was performed extracorporeally. In the second patient, the second mass was simply aspirated because adhesions were encountered. Our single-site laparoscopic-extracorporeal technique proved to be a safe approach to an otherwise high-risk situation.

Key words: Laparoscopic surgery; Pregnant complication; Ovarian mass; Fixation device; Extracorporeal

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Core tip: The new device, "Ova-Lead" has a 20-mm-diameter tip that is made of silicone and shaped like a suction cup. It fixes to the organ through the application of negative pressure, by using this device the surgeon manipulates the organ. We reported two cases of adnexal mass discovered during pregnant that this device seemed useful.

Kasahara H, Kikuchi I, Otsuka A, Tsuzuki Y, Nojima M, Yoshida K. Laparoscopic-extracorporeal surgery performed with a fixation

device for adnexal masses complicating pregnancy: Report of two cases. *World J Methodol* 2017; 7(4): 148-150 Available from: URL: <http://www.wjgnet.com/2222-0682/full/v7/i4/148.htm> DOI: <http://dx.doi.org/10.5662/wjm.v7.i4.148>

INTRODUCTION

One or more adnexal masses are discovered in a reported 0.01%-1% of all pregnancies^[1]. The majority resolve spontaneously, but in some cases, torsion or rupture necessitates emergency surgery. Ideally, surgery is performed after gestational week 12, but expansion of the uterus after the first trimester makes surgical manipulation difficult. We report adnexal masses complicating 2 pregnancies for which we used an organ fixation device for safe performance of single-site umbilical laparoscopic surgery.

CASE REPORT

Case 1

The patient was a 29-year-old woman, gravida 0 para 0. An early-stage dichorionic, diamniotic twin pregnancy was confirmed simultaneously with a right-sided adnexal mass. Pelvic magnetic resonance imaging (MRI) revealed a 60-mm mass that appeared to be a mature cystic teratoma, so laparoscopic surgery was scheduled and performed during gestational week 14. We placed Lap Protector and EZ Access (Hakko Corporation Tokyo Japan) as a wound retractor in the umbilicus, and we used an organ fixation device called "Ova-Lead" (Fuji Systems corporation, Tokyo Japan) to apply traction to the adnexa to bring the mass up to the umbilicus (Figure 1). We resected the mass extracorporeally (Figure 2). She was followed up at our hospital until 22 wk then moved overseas.

Case 2

The patient was a 38-year-old woman, gravida 0 para 0. Bilateral adnexal masses were identified during the early stage of pregnancy. Pelvic MRI revealed a 70-mm left adnexal mass and a 50-mm right adnexal mass. Both were thought to be mature cystic teratomas, so laparoscopic surgery was scheduled and performed during gestational week 14. We placed a RapidPort EZ Access Port in the umbilicus. We encountered extensive adhesions within the peritoneal cavity. Traction was applied to the left adnexa by means of an organ fixation device, "Ova-Lead" until the mass reached the umbilicus, and the mass was resected extracorporeally. Applying traction to the right adnexa proved difficult due to the adhesions, so we simply performed paracentesis. The fluid contained hemorrhagic components, so we suspected an endometrioma. The left adnexal mass was diagnosed histologically as a mixed cystic teratoma and endometrioma. No recurrence of the right mass was noted during the remaining course of the pregnancy.



Figure 1 By using "Ova-Lead", aspirate sucking and fix the ovaries to the cup.

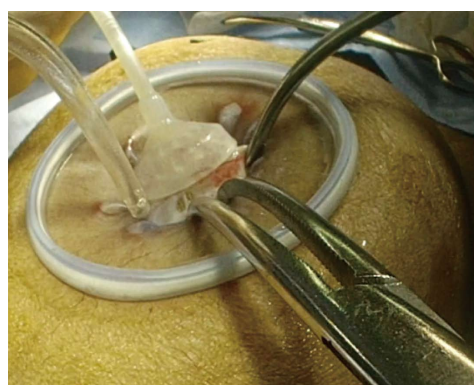


Figure 2 Ovarian cystectomy was performed extracorporeally after pulling out the whole ovary from the abdominal cavity.

She was followed up at our hospital, and at 36 wk, premature rupture of membrane occurred to her, then she was delivered vaginally.

DISCUSSION

The "Ova-Lead" has a 20-mm-diameter tip that is made of silicone and shaped like a suction cup. It fixes to the organ through the application of negative pressure (30 mmHg). The surgeon manipulates the device by a metal handle, and use of the device eases performance of the operation. At our hospital, we attach and fix the device to the adnexal mass and then apply traction to the mass to draw it up to the umbilicus. This allows us to resect the organ extracorporeally, and by using this single-port technique, the operation time is shortened, and surgery can be performed with as little leakage of tumor contents into the peritoneal cavity as possible.

Adnexal masses occurring during pregnancy are of various tissue types. The most common are mature cystic teratomas, representing 40% of adnexal masses. These are followed in order by serous cystadenomas at 20%, mucinous cystadenomas at 10%, endometriomas at 5%, and malignant tumors at 3%^[1]. The contents of a mature cystic teratoma can easily leak into the peritoneal cavity. This has been found to result in

chemical peritonitis. Therefore, at our hospital, we use an extracorporeal technique to resect the mass.

Extracorporeal resection generally reduces the overall operation time, thus shortening the pneumoperitoneum time. Jansen *et al.*^[2] verified that increases in intrauterine pressure can result in fetal hypoxia. Hunter *et al.*^[3] verified in an animal model that carbon dioxide (gas) pneumoperitoneum can cause fetal acidosis and noted that changes were greatest at pressures of 15 mmHg or more. We believe that laparoscopic-extracorporeal resection, in comparison to total intracorporeal laparoscopic resection, allows us to better shorten the duration of both the surgery and the pneumoperitoneum, helping us prevent this complication.

Adverse events can occur in pregnant women with an adnexal mass. There is potential for pedicle torsion (1%-22%), rupture (9%), miscarriage or premature labor (5%-15%), or infection (1.2%-2.4%)^[4]. The risk of such complications increases when the mass is greater than 6 cm^[4]. We recommend surgery for masses greater than 6 cm that we encounter at our hospital.

Our gynecology department guidelines recommend performing surgery after gestational week 12, after the period of organogenesis has passed. The Japan College of Radiology imaging guidelines recommend use of MRI after gestational week 14. Surgery becomes increasingly difficult with each passing gestational week, so at our hospital, we perform surgical treatment as close to gestational week 14 as possible if a diagnosis has been made by then.

In conclusion, we treated two cases of adnexal masses complicating pregnancy by performing single-port umbilical laparoscopic surgery, using an organ fixation device, and resecting the masses extracorporeally. There are risks associated with surgery performed during pregnancy, but the potential complications associated with the simultaneous presence of an adnexal mass outweigh the risks of surgery. We have found that our technique prevents the complications associated with such surgery and facilitates safe surgical treatment even when the surgical field is difficult to secure and the uterus

is especially enlarged, as in the case of a twin pregnancy. And also, even in the case of a large ovarian tumor, this method was suggested to be useful.

ARTICLE HIGHLIGHTS

Clinical diagnosis

Ovarian cyst (benign).

Differential diagnosis

Ovarian carcinoma, etc.

Imaging diagnosis

Magnetic resonance imaging findings as follows: Case 1: Mature cystic teratoma; Case 2: Endometrioma.

Pathological diagnosis

Case 1: Mature cystic teratoma; Case 2: Endometrioma.

Treatment

Surgical treatment.

Related reports

This manuscript was the first report about this device.

Experiences and lessons

In the case of a large ovarian tumor, this "ova-lead" was suggested to be useful.

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