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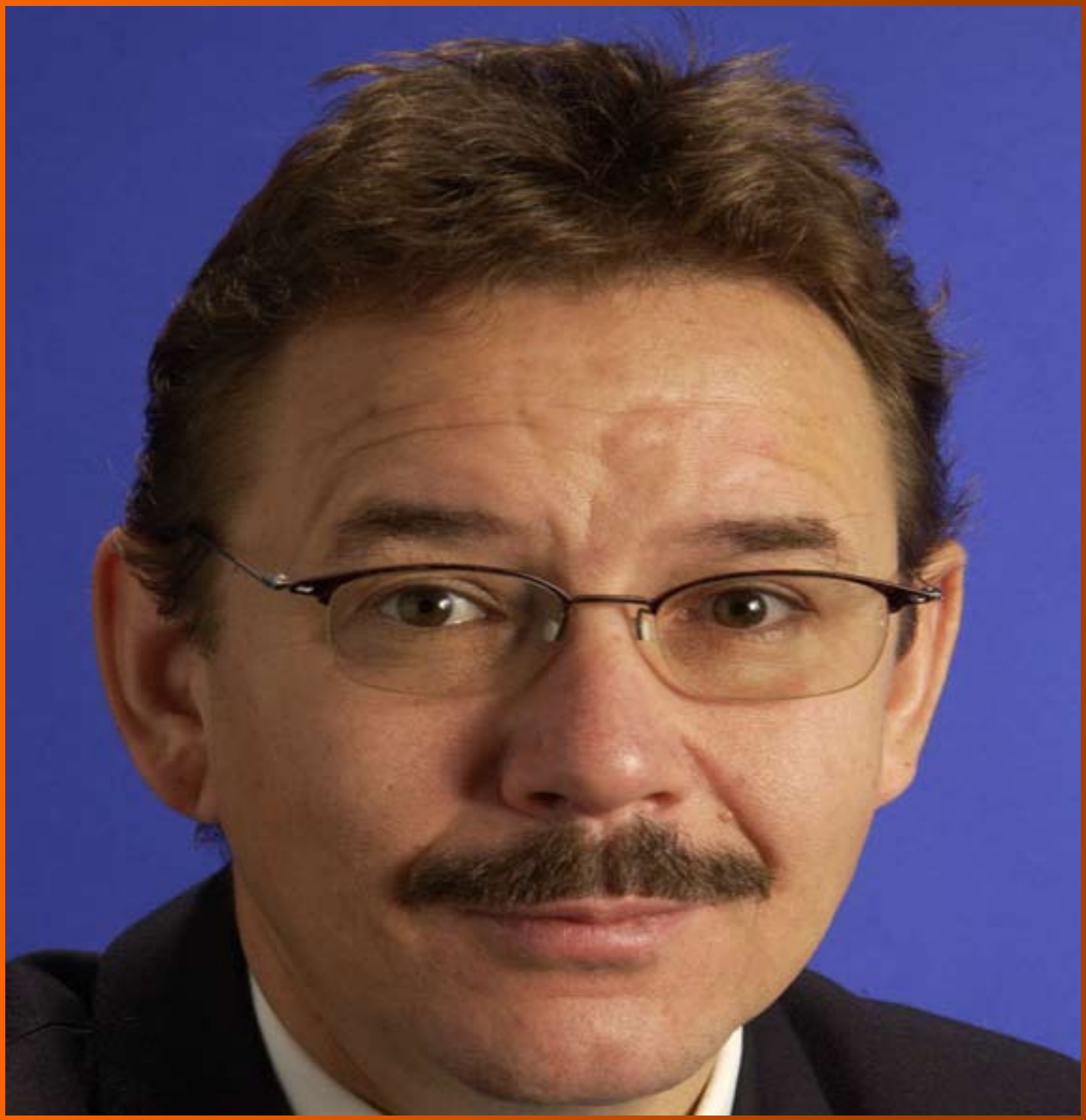

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MINIREVIEWS

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; Autoimmune diseases; Laboratory biomarkers; Diagnostic markers

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 composit 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-fliaggrin, 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 immunoflourescent (IF) techniques are used. Together with the variationin 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. Negatif 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 carefull 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 normogram (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

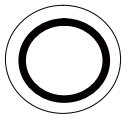
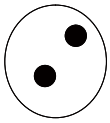
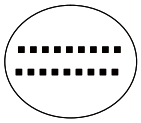
ANA pattern	Antigen	Associated diseases
Speckled 	ENA, RNP, Sm, Ro/SSA, La/SSB, Scl-70, Jo-1, ribosomal-P	SLE, MCTD, systemic sclerosis, Sjögren's syndrome, PM
Homogenous 	dsDNA, Histones SLE	Drug-induced SLE
Peripheral (rim) 	RNP, Sm, Ro/SSA SLE	Systemic sclerosis
Nucleolar 	Anti-PM-Scl, anti-RNA polymerase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

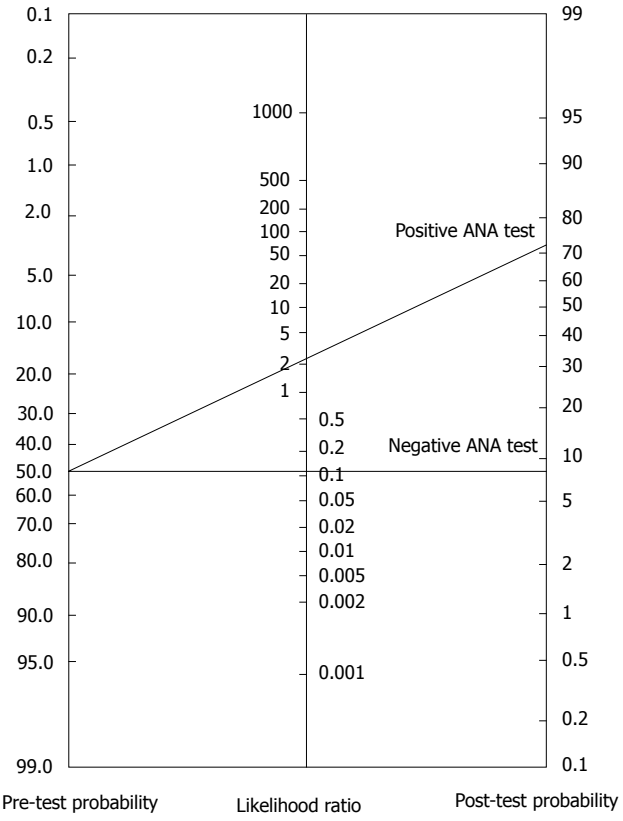


Figure 2 The likelihood nomogram used in systemic lupus erythematosus with an antinuclear antibody test.

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 (ANCAs) 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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MINIREVIEWS

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

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 HISL-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 Catestin, 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 $\geqslant$ 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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ORIGINAL ARTICLE

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-respondent 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

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.

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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 $CHADS_2$ 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	Branching survey logic mapped score calculators to applicable specialties
	Survey distribution	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	*n* 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%)
Hemorr2hages 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	
n 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	
n 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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ORIGINAL ARTICLE

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

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.

Shiny TN, Mahajan VK, Mehta KS, Chauhan PS, Rawat R, Sharma R. Patch testing and cross sensitivity study of adverse cutaneous drug reactions due to anticonvulsants: A preliminary report. *World J Methodol* 2017; 7(1): 25-32 Available from: URL: http://www.wjgnet.com/2222-0682/full/v7/i1/25.htm DOI: http://dx.doi.org/10.5662/wjm.v7.i1.25

INTRODUCTION

Adverse cutaneous drug reaction (ACDR) is a frequent problem in clinical practice comprising 1%-2% of outdoor and 6%-30% of indoor patients in dermatology. ACDRs from anticonvulsants [carbamazepine, phenytoin, phenobarbitone (aromatic group), lamotrigine and sodium 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 $\geqslant$ 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 Ardern-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 *n* = 24 (%)
Gender	
Male	13 (54.2)
Female	11 (45.8)
M:F	1:1.8
Age (yr)	
Range	18-75
Mean ± SD	45.70 ± 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 ± SD	22.54 ± 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 ± SD	9.62 ± 6.62

[1]Also 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 (*n* = 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 valporate 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-

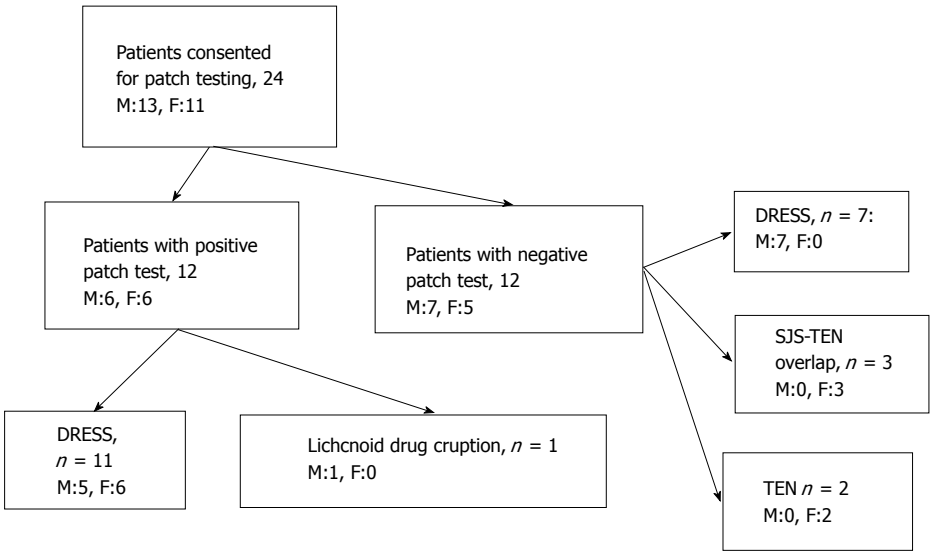

Figure 1 Attributes of 24 study patients for drug patch testing at a glance.

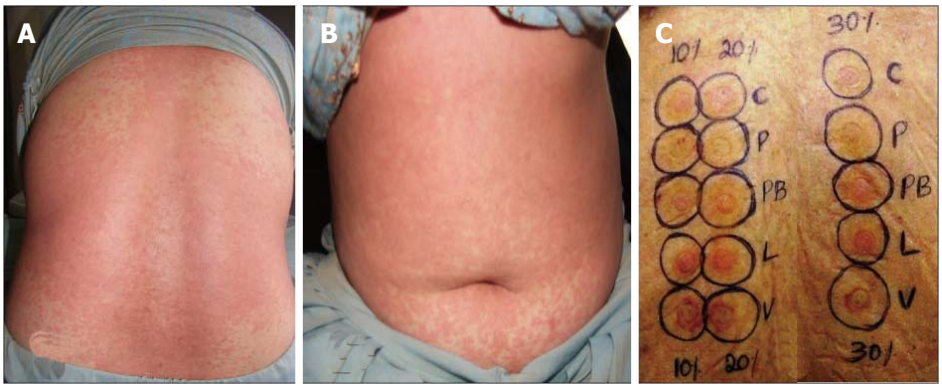

Figure 2 A patient of drug rash with eosinophilia and systemic symptoms from carbamazapine having prominently macular erythema and pruritic maculopapular rash over trunk (A) back, (B) front. She also had facial edema, conjuctival congestion and chemosis (not in picture); (C) Drug patch tests reactions (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 ACDRs 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 ACDRs. Frequency of positive drug patch tests varies between 7% and 87% in ACDRs 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/exanthematos 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	32F	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 ACDRs 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 effecter 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-Tc1cells) 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 erytematous 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; Exanthemataous 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 AGEP (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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