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Primary immune thrombocytopenia: a 'diagnosis of exclusion'?

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Current diagnosis of primary immune thrombocytopenia (ITP) is presumptive, centered on excluding other causes of thrombocytopenia. The diagnosis of ITP is challenging because of the wide range of potential inherited and acquired causes of thrombocytopenia. The treatment of ITP is empiric with steroids, high-dose immunoglobulin, immunosuppressants and thrombopoietin agonists with potential side effects. We searched Medline and Cochrane databases, reviewed the study data and analyzed the individual diagnostic tests for their evidence-based role in the diagnosis of ITP. We then analyzed the strength of the scientific evidence for each diagnostic test in the diagnosis of ITP and identified gaps in the diagnostic accuracy. The diagnostic challenges in ITP include: insufficient evidence for the individual test for diagnosis of ITP, no standardized protocol/guideline for diagnosis, hurdles in accessing the available resources and failure to correlate the clinical data while reviewing the blood smear. We did not identify a diagnostic test that clinicians can use to confirm the diagnosis of ITP. In the absence of a diagnostic test of proven value in ITP, the clinician is best served by a comprehensive history and physical examination, complete blood count and review of the peripheral blood smear in evaluating thrombocytopenia. *Blood Coagul Fibrinolysis* 33:289–294

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Introduction

Primary immune thrombocytopenia (ITP) is an acquired immunological disorder defined as thrombocytopenia less than 100×10^9/l with no underlying cause [1]. In the general medical practice, ITP is the most common cause of severe isolated thrombocytopenia (80%). The incidence of ITP ranges from 2 to 4 cases per 100 000 person-years, with a peak incidence between 20 and 30 years of age, with a slight female predominance and a larger peak above 60 years of age with an equal sex distribution [2]. The term idiopathic thrombocytopenic purpura was changed to immune thrombocytopenia and later to primary immune thrombocytopenia in recognition of the pathogenesis and to exclude other causes of thrombocytopenia [3,4].

Devoid of a definitive confirmatory test, it is difficult to reliably establish the diagnosis without excluding congenital and acquired causes. Congenital thrombocytopenia may masquerade as ITP. Hence, without historical platelet counts from the patient or family members, ITP may be difficult to diagnose. Primary immune thrombocytopenia may also be the initial manifestation of acquired autoimmune disorders including systemic lupus erythematosus (SLE), wherein 20–30% of patients develop ITP during the course of the disease [5]. The clinical course of ITP may be very heterogeneous. Although most of the childhood ITP remit spontaneously without complications, some children bleed and develop a chronic protracted illness. Adults with ITP may enter a deep remission with a short course of corticosteroids alone or suffer a chronic relapsing disease [6,7].

Since the original description by Harrington and James W. Hollingsworth in 1950, there has been no significant advancement made in the diagnostic criteria, despite the fact that rapid strides have been made in the management of ITP [8]. In practice, after initial suspicion of ITP and detailed clinical evaluation, appropriate tests to exclude a secondary cause is done before treatment is initiated [9]. Our aim is to study the diagnostic tests available for ITP and analyze the evidence for each of them in support of the diagnosis of ITP.

Methods

We used Medline and Cochrane databases through August 2021 and found 3797 studies reporting data on the diagnosis of ITP. To be considered for this review, the inclusion criteria encompassed the following: the article must be on primary ITP, the diagnostic test should be unequivocal in the diagnosis and the article had to deal explicitly with the diagnosis of ITP excluding other causes confounding

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the diagnosis. Thus, we excluded articles pertaining to thrombocytopenia including secondary ITP, inherited disorders, medication, herbal mediated, primary marrow disorders, sequestration induced and other disorders associated with hemostasis. We then selected diagnostic tests and analyzed their individual merits in support of the diagnosis of ITP.

Data extraction

Randomized controlled trials, case series and abstracts published in peer reviewed journals presented in national and international meetings were reviewed. Data was extracted on author names, location, specific intervention, comparison details, outcomes and participants according to the *Cochrane Handbook for Systematic Reviews of Interventions*. Two authors (N.V. and R.K.) in duplicate reviewed the titles, abstracts, and full texts. Standardized data extraction form was used for data collection. Conflicts were resolved by consensus through consultation with other authors. Additional references were obtained from references retrieved by the manual search and from the bibliographies of the retrieved articles.

Results

Peripheral blood smear

Whenever ITP is suspected, the peripheral blood smear is examined to exclude other causes of thrombocytopenia including pseudothrombocytopenia. The blood smear is reviewed for abnormal red blood cells (schistocytes), white cells (blasts, toxic granulation of polymorphs, Döhle bodies) and platelets (clumping of platelets, rosette formation) prior to the diagnosis of ITP [10]. Congenital thrombocytopenia may be identified by changes in platelet morphology (giant platelets, gray platelets) [11]. The interpretation of blood smear may require expertise and easy access to microscopy. Digital microscopes allowing transmission of images to experts in remote locations and computer-assisted digital microscopes for evaluation of blood films are under development. Apart from the paucity of platelets, there is no diagnostic marker for ITP in peripheral blood smear.

Mean platelet volume

This parameter suggests the bone marrow's response to platelet sequestration in ITP (normal range: 7.5–11.5 fl). An increased mean platelet volume is typically seen in the context of bone marrow response to increased platelet sequestration in ITP but this may also be seen in inherited thrombocytopenia. It is a machine-calculated ratio of plateletecrit and platelet count. The highest values are seen in ITP [12]. Another study found that the MPV larger than 12.4 fl can differentiate inherited macrothrombocytopenia as in MYH9-related disease and Bernard–Soulier syndrome from ITP with a sensitivity and specificity of almost 90% making MPV a useful parameter for differentiating inherited from immune thrombocytopenia [13]. In a study of 171 patients with thrombocytopenia, 4 out of 37 patients with ITP, the MPV was not elevated, though the highest values were found in ITP with a P value of less than 0.0001 [12]. The MPV in patients with ITP and healthy population was not very different (P value 0.76) but high MPV had better outcome in patients with ITP [14]. The MPV cannot be measured accurately with very low platelet count as the analyzer may be unable to derive the MPV because of the platelet histogram distribution. Therefore, MPV though statistically capable of differentiating disorders of thrombocytopenia, cannot be considered a diagnostic marker for ITP, especially when platelet count is extremely low.

Immature platelet fraction

Immature platelet fraction (IPF) gives an indirect determination of platelet production. The IPF is usually elevated in ITP [15]. The immature platelets circulating in the blood contain residual RNA, giving an indirect determination of platelet production. The IPF is detected by flow cytometry. This may be a practical tool to differentiate thrombocytopenia of decreased production from increased destruction [16]. In a study using IPF, the mean IPF % value by Sysmex XE-2100 was found useful to predict ITP. In a study of 231 patients, 62 were diagnosed as ITP and 169 as non-ITP, the mean IPF % value for ITP was 16.39% compared with 7.69% for non-ITP patients [17]. In a study comparing 41 patients with ITP and 14 patients with thrombocytopenia from hematological malignancies on chemotherapy, the authors found the IPF to be a rapid and inexpensive automated marker for discerning the cause of thrombocytopenia. They concluded that IPF may also be useful as a potential prognostic marker for chronic ITP. The median IPF was 11.8% in patients with ITP, 7% in those with hematological malignancy and 3% in the control group ($P < 0.001$) [18]. The IPF may also be used to assess the response to treatment in ITP [19]. However, elevated IPF demonstrates rapid turnover of megakaryocytes within the bone marrow and is not specific for an underlying immune-mediated mechanism [20]. A lack of standardization of methods and definition of threshold values make the interpretation of IPF difficult [19].

The diagnostic utility of platelet-specific autoantibody assays

A systematic review of platelet GP IIb/IIIa and/or GP Ib/IX-specific glycoprotein-specific platelet autoantibody testing analyzed 1170 ITP patients, and 225 nonimmune thrombocytopenic controls. The glycoprotein-specific autoantibody assay sensitivity was low (50%), whereas the specificity was high (90%). The possible explanations for this low sensitivity and high specificity in ITP may be that a proportion of ITP patients have autoantibodies against other nonplatelet target antigens, such as thrombopoietin or its receptor c-Mpl, or the autoantibodies are undetectable in some patients (because of low titer or sequestration). In children with ITP platelet GP IIb/IIIa

was positive in 72% [21]. There may be other pathological immune mechanisms present that are independent of platelet autoantibodies, such as cytotoxic T cells [22]. Assays for antibodies to specific platelet glycoproteins are not routinely recommended as platelet-associated IgG is elevated in both immune and nonimmune thrombocytopenia. This suggests that ITP is a heterogenous group of disorder caused by multiple mechanisms including, but not limited to, antiplatelet autoantibodies [23].

Other serological tests

Testing for antinuclear antibody (ANA) or other serological tests are not recommended in children and adults with suspected ITP. The antiphospholipid antibodies (APLA), including anticardiolipin antibodies are found in about 40% of patients presenting with ITP [24,25]. The presence of APLA does not appear to affect the response to treatment in ITP [26]. A positive ANA test may be a predictor of chronicity of ITP [27]. Antithyroid antibody was seen in 8–14% of ITP patients who developed clinical hyperthyroidism [28]. Overall, direct antiglobulin testing or testing for ANA, antiphospholipid antibodies, antithyroid antibodies, or thyroid function tests may exclude other causes of thrombocytopenia when clinical suspicion exists [29,30].

Refractoriness of platelet transfusion in the diagnosis

Patients with ITP are considered to have relative refractoriness to platelet transfusion because of platelet-specific immunoglobulins. Studies have tried to quantify the refractoriness by the 10 min postinfusion-corrected count increment (CCI) and the percentage platelet response (PPR), but this topic remains debatable [31]. The time to reach total body equilibrium for transfused platelets varies considerably between normal volunteers and thrombocytopenic patients [32]. A CCI of less than 7500 or a PPR of less than 30% have been used to define refractoriness. However, the evidence to support this concept is highly variable [33]. Confounding nonimmune factors associated with refractoriness frequently include fever, infection and medications [33]. Hence, platelet refractoriness cannot be used for the diagnosis of ITP.

Bone marrow examination

In a study of 296 cases of childhood ITP, none had a change of the diagnosis after the bone marrow examination [34]. In adults, bone marrow examination did not change the diagnosis of ITP when there was isolated thrombocytopenia [35,36]. In a prospective study of 353 cases, bone marrow examination in ITP revealed normal hematopoietic elements. The authors in this study concluded that routine bone marrow examination may not be required in the diagnostic work-up of ITP [37]. Bone marrow examination is not recommended for routine evaluation in patients with typical ITP at presentation [29,38,39]. However, bone marrow examination must be considered in patients older than 60 years, those with atypical or specific clinical or hematological features, those with a poor response to standard therapy with steroid, or patients in whom splenectomy is contemplated [36,40].

Genomics in the diagnosis

Sequencing of relevant pathological genes known to cause thrombocytopenia may be an important diagnostic approach in ITP. As genetic studies for inherited causes of thrombocytopenia becomes more readily available, rates of detection and diagnosis of nonimmune platelet disorders will increase and be further characterized [41]. A thrombogenomics platform correctly identified the pathogenic variants in all 159 samples with a known inherited platelet dysfunction demonstrating an impressive sensitivity of 100% [42]. Whole exome and genome sequencing has established a number of genetic causes for thrombocytopenia [43]. This may help to differentiate congenital causes of thrombocytopenia from ITP. In patients with refractory ITP with atypical features, alternative causes of thrombocytopenia should be considered. Several well characterized platelet genes encode the most abundant platelet-specific proteins including glycoprotein Ibα, glycoprotein IIb and platelet factor 4. Abnormalities of the genes may help to delineate the platelet function and immunological status of the platelets [44].

The role of thrombopoietin

Patients with ITP have normal or only slightly elevated thrombopoietin levels. In contrast, patients with other causes of thrombocytopenia, in particular, aplastic anemia, have markedly elevated levels of thrombopoietin (TPO) [45]. A prospective screening study of endogenous thrombopoietin levels in 205 ITP subjects showed no significant difference between circulating concentrations of TPO level in control versus patients with ITP [45]. Routine testing for TPO levels is not recommended for diagnostic purposes [46]. The role of serum TPO in predicting response to thrombopoietin receptor agonist (TPO-RA) therapy is under research [1,29].

Diagnostic difficulties in immune thrombocytopenia

Establishing the diagnosis of ITP in most patients seems relatively straightforward for experienced physicians but standardization of the diagnosis of ITP remains challenging. In our search, we found no prospective or retrospective study unequivocally confirming the diagnosis of ITP (Table 1). The need to identify secondary causes is crucial for definitive therapy and lack of agreement in the diagnostic criteria can be challenging [47]. When a panel of three hematologists provided their opinion on thrombocytopenia by reviewing case reports, there was discrepancy in the diagnosis of ITP among panelists [48].

One study showed improved agreement in the diagnosis of ITP when the following criteria were present: a low platelet nadir ($<20 \times 109$/l) and a platelet count increase

Table 1 Studies analyzing different parameters in immune thrombocytopenia

Author	Study type	Patients	Age group	Parameter	Confirms ITP	Comment
Akkuş *et al.* [14]	R/P	70	Adults	MPV	No	Good prognosis if MPV is high
Jeon *et al.* [20]	R/P	568	Adults	IPF	No	Elevated in ITP. Cannot be used as a sole criterion
Schmidt *et al.* [21]	P/P	179	Children	GPIIb/IIIa	72%+ve	Prognosis is good if GPIIb/IIIa is positive
Porcelijn *et al.* [46]	P/P	72	Children/adults	TPO	No	Not useful in diagnosis of ITP
Abdurrahman *et al.* [38]	R/P	122	Children	Bone marrow	No	Not useful in diagnosis of ITP
Gunduz *et al.* [39]	R/P	98	Adults	Bone marrow	No	Not useful in diagnosis of ITP

ITP, immune thrombocytopenia; MPV, mean platelet volume; P/P, prospective; R/P, retrospective; TPO, thrombopoietin.

following treatment with intravenous immunoglobulin (IVIG) or corticosteroids but this will not exclude secondary ITP [48]. A case series found 15% of ITP patients fulfilling the diagnosis of SLE on detailed evaluation [49]. Up to 10% of patients with ITP test positive for antiphospholipid antibodies and/or lupus anticoagulant [50]. About 5–10% of patients with lymphoproliferative disorder and 10% with common variable immunodeficiency develop ITP [51]. In a large, prospective, multicenter, international registry over 15 years, 3974 children and adolescents with an initial diagnosis of primary ITP were analyzed, revisions to the diagnosis were made in 241 children within 24 months of follow-up. Ultimately, 113 patients had an unequivocal diagnosis of secondary ITP [52]. Another study found that 10 (13%) of 75 ITP patients had positive serologic findings for Epstein–Barr virus, cytomegalovirus, or rubella virus-associated immune thrombocytopenia in childhood [53]. Contrary to the expectation, initial platelet counts and the number of patients with profound thrombocytopenia ($<20 \times 10^9$/l) were not significantly different between secondary and primary ITP [54,55].

Caution in accepting immune thrombocytopenia as a diagnosis of exclusion

The diagnosis of ITP needs great caution and follow-up as this may convert to other immune-mediated disorders over time [56]. One study estimated that the diagnosis of ITP was inaccurate among one in seven patients suspected of having ITP [57]. Response to ITP-specific therapy including intravenous immunoglobulin (IVIg), intravenous anti-D, steroid therapy, rituximab and splenectomy appears to be the single most diagnostic criterion [1]. However, response to these agents may occur in other conditions. In a study of 492 patients who had the diagnosis of ITP, 17% were found to have alternative diagnoses on chart review, with coding classification errors in 3%, or an alternative explanation for their thrombocytopenia consisting of 31 different diagnoses in 14%. The most common diagnoses were familial thrombocytopenia (10%), systemic lupus erythematosus (9%), hypersplenism (9%), neonatal alloimmune thrombocytopenia (7%), Wiskott–Aldrich syndrome (7%) or systemic infection (6%). In total, 16 patients (23% of the alternative diagnosis and 3% of the total population) were ultimately diagnosed with inherited platelet function disorders [58].

There are algorithms published in the medical literature to help with the diagnosis of ITP. These algorithms are aimed to describe, which test/parameter should be undertaken in case of chronic ITP but do not appear to be informative [30,59]. Some investigators have suggested that a progressive algorithm with further investigations to be performed in patients with persistent and chronic ITP. However, these algorithms are driven by the specific investigations performed and not by the possible differential diagnosis [60].

The heterogeneity of ITP is demonstrated by the bleeding symptoms. Most patients with chronic ITP with low platelet count do not bleed [4]. Some with higher platelet counts bleed spontaneously and dangerously. There is no direct correlation with the platelet count and the severity of illness in ITP. When measured systematically using an ITP-specific bleeding tool, a prospective study suggested that the frequency of grade 2 bleeding is higher than previously reported [57]. A systematic review found that 56% of patients with primary ITP experienced severe bleeding at some point during their disease course. This estimate is higher than what has been previously reported in the medical literature. Severe bleeding occurred in approximately 10% for adults out of which intracranial hemorrhage accounted for 1–1.5% of the cases [61]. In a retrospective study with 13 064 patient-years of follow-up, 3768 patients (57%) experienced at least one bleeding-related events per patient-year. The majority (58%) of bleeding-related events were treated with rescue therapy. Common bleeding categories included gastrointestinal hemorrhage, hematuria, ecchymosis and epistaxis [62]. History of mucocutaneous bleeding cannot be used to differentiate ITP from other causes of thrombocytopenia.

Limitations

This review has certain limitations, including the small sample size of the included studies. There was lack of standardization of methods and consistency of the tests. This hinders meaningful assessment of test results. Due to the nature of the search methodology used, some literature may have been inadvertently omitted from the conferences outside of Europe and America as well as unpublished articles. The number of studies were limited to reporting and publication bias. The study is also limited by challenges of an on-line literature search.

Conclusion

The diagnosis of ITP continues to be presumptive and empirical. As there is no diagnostic test for ITP, the clinician is best served by a comprehensive physical examination, examination of the peripheral blood smear (for morphological abnormalities) and assess initial treatment response. When patients fail initial therapy, bone marrow examination, auto-immune serology (to rule-out an underlying connective tissue disorder), iatrogenic and infectious disease evaluations should be considered, unless an obvious cause for the thrombocytopenia becomes evident. Identifying the underlying cause of thrombocytopenia is crucial for the appropriate management. There appears to be no agreement regarding the tests needed to complete the diagnostic work-up. Future research in diagnostic testing for ITP is needed to prevent morbidity. Identifying the underlying cause of thrombocytopenia is crucial for the management of ITP

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Data availability: the authors declare that data supporting the findings of this study are available within the article.

Conflict of Interest

There are no conflicts of interest.

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