Imaging techniques for the diagnosis of pulmonary embolism

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Abstract: Pulmonary embolism (PE) is a cardiovascular emergency with high morbidity and mortality. The diagnostic workup of patients with suspected acute PE remains difficult due to a wide spectrum of clinical presentations. There is still no diagnostic test that is accurate, safe, readily available, and cost-effective. Pulmonary angiography has a high diagnostic accuracy, but it is an invasive and resource-demanding procedure. Noninvasive imaging tests including computerized tomographic pulmonary angiography and ventilation/perfusion scanning are well validated for the diagnosis of PE, but have limited sensitivity and specificity. For optimal efficiency, the choice of the initial imaging modality should be guided by the clinical probability assessment and D-dimer testing. This review covers the performance of different diagnostic tests and presents a diagnostic algorithm for PE diagnosis.

Keywords: pulmonary embolism, diagnosis, algorithm, imaging, cardiovascular, mortality

Introduction

Acute pulmonary embolism (PE) is one of the most common cardiovascular emergencies and is the main cause of death in hospitalized patients older than 65 years.¹ The annual incidence of PE has been reported to be around 1/500 persons, but the true incidence is likely to be larger due to nonspecific clinical presentations of PE.²⁻⁴ Acute PE spans a wide clinical spectrum with largely nonspecific signs and symptoms. Therefore, the diagnostic workup of patients with clinically suspected PE is challenging and remains a major clinical problem.⁵ Since early initiation of antithrombotic therapy in patients with proven PE highly improves survival, immediate and accurate diagnostic tests are of great clinical relevance.⁵ The optimal diagnostic imaging modality for the diagnosis of PE continues to be debated. There are many types of imaging techniques that have found an application in patients with clinical suspicion of PE, including computerized tomographic pulmonary angiography (CTPA), ventilation–perfusion (V/Q) scanning, conventional pulmonary arteriography, echocardiography, magnetic resonance imaging (MRI), and imaging for deep vein thrombosis as an origin for acute PE. At present, CTPA is used in the vast majority of patients with suspected PE. Conventional pulmonary angiography (PA) is still the reference standard for PE diagnosing. As imaging tests with a 100% sensitivity and specificity for acute PE are not available, limitations can be better managed by combining imaging techniques, clinical probability assessment, and D-dimer testing.

This review focuses on currently available and validated imaging techniques for the diagnosis of PE. A straightforward diagnostic algorithm is then presented. Newer
imaging techniques such as helical CT and MRI are not evaluated because of the limited data currently available.

**Imaging techniques**

**Chest radiography**

Chest radiography in patients with PE is usually abnormal. Common radiographic findings include plate-like atelectasis, pleural effusion, pulmonary infiltrates, and elevation of a hemidiaphragm. However, these signs are nonspecific. Classic signs of pulmonary infarction such as Hampton’s hump (wedge-shaped consolidation in the lung periphery) or Westermark’s sign (local oligemia) are suggestive but infrequent. In general, chest radiography cannot be used to diagnose or exclude PE. As an initial diagnostic test, its main value is in the differential diagnosis of other cardiorespiratory diseases, which mimic the clinical presentation of PE, such as pneumonia, pneumothorax, rib fracture, or congestive heart failure.

The advantages are that chest radiography excludes other causes of dyspnea and chest pain, and the disadvantage is that encountered findings are nonspecific.

**Computerized tomographic pulmonary angiography**

CTPA is increasingly being used as the main thoracic imaging test for the evaluation of PE. The well-recognized advantages include speed, wide availability, ability to define associated or alternative diagnoses, direct visualization of the thrombus, and the high number of definitive diagnostic results (either positive or negative) (Figure 1). Data from several studies comparing the performance of the first-generation single-detector (SD) CTPA with pulmonary arteriography reported wide variations in both sensitivity (53%–100%) and specificity (81%–100%) of SD-CTPA. In two large prospective studies, SD-CTPA had an overall sensitivity for PE of around 70% and specificity of 90% and is, therefore, considered as too insensitive to be used as a single test for ruling out PE. Furthermore, the sensitivity proved to depend on the location of PE with a sensitivity of 86% for segmental or larger PE and merely 21% for subsegmental PE. Two large outcome studies stressed the importance of additional ultrasonography in case of a negative SD-CTPA. With the emergence of multidetector-CTPA (MD-CTPA), CTPA has gained substantially in scanning speed and resolution allowing decreased section thickness, reduced scanning times, and adequate visualization of pulmonary arteries up to segmental and subsegmental vessels. Comparing the diagnostic accuracy of MD-CTPA with that of PA, a sensitivity and specificity for PE of above 90% have been reported. However, the overall diagnostic sensitivity of MD-CTPA reported in the large multicenter Prospective Investigation of Pulmonary Embolism Diagnosis (PIOPED) II study was only 83% (95% confidence interval, 76%–92%). This study emphasized the influence of objective clinical probability, as assessed by the Wells criteria (Table 1) to rule out PE. The predictive value of MD-CTPA was high with an 89%–95% negative predictive value (NPV) and a 92%–96% positive predictive value (PPV) in patients with a concordant clinical assessment, whereas it was lower (NPV 60% and

![Figure 1 Diagnosis of PE with CT. 3D reconstruction of CT angiography reveals that the right inferior pulmonary artery with its side branches is almost completely absent due to thromboembolic occlusion. Dotted circle marks the area of hypoperfusion. Abbreviations: 3D, three-dimensional; PE, pulmonary embolism; CT, computed tomography.](image-url)

**Table 1** Wells rules for clinical risk stratification in patients with suspected PE

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical signs and symptoms of deep vein thrombosis</td>
<td>3</td>
</tr>
<tr>
<td>PE as likely or more likely than an alternative diagnosis</td>
<td>3</td>
</tr>
<tr>
<td>Heart rate &gt;100 beats/minute</td>
<td>1.5</td>
</tr>
<tr>
<td>Immobilization or surgery in the previous 4 weeks</td>
<td>1.5</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>1</td>
</tr>
<tr>
<td>Malignancy</td>
<td>1</td>
</tr>
<tr>
<td>Clinical probability (extended)</td>
<td>Total</td>
</tr>
<tr>
<td>Low</td>
<td>0–1</td>
</tr>
<tr>
<td>Intermediate</td>
<td>2–6</td>
</tr>
<tr>
<td>High</td>
<td>≥7</td>
</tr>
<tr>
<td>Clinical probability (simplified)</td>
<td></td>
</tr>
<tr>
<td>PE likely</td>
<td>0–4</td>
</tr>
<tr>
<td>PE unlikely</td>
<td>&gt;4</td>
</tr>
</tbody>
</table>

**Abbreviation:** PE, pulmonary embolism.
PPV 58%) when clinical probability was inconsistent with the imaging result.

Recent studies suggest that MD-CTPA can reliably be used as the single imaging test for suspected PE in patients with low or intermediate clinical PE probability. The combination of clinical probability, D-dimer testing, and MD-CTPA proved to be safe for ruling out PE in several large prospective management studies. The 3-month risk for venous thromboembolic (VTE) events in patients who were left untreated after a negative MD-CTPA was 0.6%–1.5%. For comparison, conventional PA has a 3-month VTE risk rate of around 1%–2%. Furthermore, the algorithm allowed a management decision in 98% of patients. As further evidence, two randomized trials suggested very low additional yield from confirmatory imaging of the leg veins in patients with normal MD-CTPA. The most recent study demonstrated that a strategy using D-dimer and MD-CTPA is equally safe as using D-dimer, venous ultrasonography of the leg, and MD-CTPA. The 3-month VTE rates were 0.3% in both groups.

False-negative MD-CTPA results are most often related to subsegmental thrombi. Indeed, the clinical relevance of isolated subsegmental thrombi is controversial. However, additional testing (eg, V/Q lung scanning) should be considered in patients with a high clinical probability of PE. False-positive CTPA results appear to be unusual. Limitations of CTPA include cost, relatively high radiation exposure, and contraindications to iodinated contrast material in patients with reduced renal function or iodine allergy. The radiation dose from MD-CTPA has been recently identified as an important public health problem especially in young women.

The advantages of CTPA include speed, wide availability, adequate visualization of pulmonary arteries up to segmental and subsegmental vessels, definitive diagnosis of PE (either positive or negative), and the ability to establish alternative diagnoses. The disadvantages of CTPA include cost, high radiation exposure, and the possibility of inducing nephropathy or allergy associated with iodinated contrast material.

Computational tomography venography

The diagnostic value of additional computed tomography venography (CTV) in suspected PE was investigated in the PIOPED II study. Indeed, the combination of MD-CTPA and CTV revealed a higher sensitivity (90%) for the diagnosis of PE than CTPA alone and a similar specificity (96% for CTPA alone and 95% for CTPA + CTV). However, the increase in NPV was less (97% vs 96%). Since additional CTV increases radiation exposure without yielding significantly different predictive values compared with CT alone, this procedure is not recommended as routine diagnostic.

The advantages are that combining CTPA with CTV increases sensitivity, but the disadvantage is that the absolute gain due to CTV is modest, and the overall radiation during examination is increased.

V/Q scanning

The basic principle for the diagnosis of PE based upon V/Q scanning is to recognize lung segments or subsegments without perfusion but preserved ventilation (called mismatch). In Germany, ventilation studies are usually carried out with radioaerosols or with Technegas (Cyclopharm, Melbourne, Australia). Technegas is an aerosol comprising Tc-99m-labeled carbon microparticles generated at high temperature, which have a diameter of about 0.005–0.2 µm. The use of Tc-99m-Technegas has minimized the problem of hot spots in patients with obstructive lung disease and is according to clinical experience better than the

![Figure 2 Diagnosis of pulmonary embolism with scintigraphy. A) Coronal ventilation and B) coronal perfusion scintigraphy displays perfusion defects with intact ventilation (dotted circles) as a sign of pulmonary embolism.](image-url)
best liquid aerosols. Perfusion studies are performed after intravenous injection of macroaggregated human albumin (MAA).

**V/Q planar**

Since the publication of the first PIOPED study in 1990, many lessons have been learned concerning V/Q scanning. Detection of ventilation and perfusion defects at the subsegmental level is possible by planar imaging (V/Q planar) but is considerably better by V/Q-single photon emission computed tomography (SPECT). Poor results that were attributed to the performance of V/Q scanning can be in part explained by the use of planar images in the PIOPED studies. Follow-up studies provided a more detailed analysis on PIOPED data. More than 10 years after the publication of the first PIOPED study, V/Q scanning was validated in relation to a true gold standard using artificial subsegmental emboli. The sensitivity of V/Q planar was 67%, while V/P-SPECT performed much better with 93%. In clinical studies, Bajc et al identified 53% more mismatched regions with V/Q-SPECT than with V/Q planar.

**V/Q-SPECT**

Nowadays, SPECT is the standard for how to perform V/Q studies (Figure 3), and SPECT-CT is the next diagnostic step. In addition to the above-mentioned study from Bajc et al, Collart et al showed that V/P-SPECT increased the specificity for PE from 78% to 96% at similar sensitivities. Reinartz et al found a sensitivity and specificity of 76% and 85%, respectively, with V/Q planar compared with 97% and 91% with V/Q-SPECT. In a head-to-head comparison, Gatte et al compared the performance of V/Q-SPECT with low-dose CT and MD-CT and found a sensitivity, specificity, and accuracy of 97%, 88%, and 91%, respectively, for V/Q-SPECT; 68%, 100%, and 88%, respectively, for MD-CT, and 97%, 100%, and 99%, respectively, for V/Q-SPECT/low-dose CT. Interpretaion criteria for V/Q scanning

Probabilistic interpretation that was used in the PIOPED I and II studies were either never accepted in Germany or have been abandoned nowadays. An important step in improved reading of perfusion scans is the Prospective Investigative Study of Acute Pulmonary Embolism Diagnosis (PISA-PED) study. By using clinical evaluation and by recognition of perfusion patterns typical of PE, the number of nondiagnostic examinations decreased significantly. According to contemporary understanding, holistic interpretation of scintigraphic images includes clinical information and laboratory test together with all observed signs and patterns on ventilation and perfusion scintigrams. Schemes for clinical probabilities may be of significant value. Applying holistic principles of interpretation of V/P-SPECT, recent studies have shown NPVs in the range 97%–99%, sensitivities in the range 96%–99%, and specificities in the range 91%–98% for the diagnosis of PE. Rates of nondiagnostic findings were 1%–3%. Furthermore, the NPV of V/Q scanning for recurrent PE is unsurpassingly high.

Allergic reactions to Tc-99m-MAA used for lung perfusion imaging are very rare, and radiation exposure of V/Q scanning is much lower in comparison to CT. According to the International Commission on Radiological Protection (ICRP), the effective radiation dose of V/Q imaging is 1.8–2 mSv. Radiation exposure for the female breast is in the range of 0.8 mSv and about a factor of 10 lower than CT.
The advantages of V/Q scanning are that it carries no contraindications and gives a low radiation burden. V/Q-SPECT has a high sensitivity and specificity for PE. However, the disadvantages are that V/Q planar findings are frequently inconclusive. V/Q-SPECT is in general less readily available.

**Pulmonary angiography**

For many decades, PA was regarded as the gold standard imaging technique for the diagnosis of PE with a sensitivity of around 98% and specificity between 95% and 98%. The two definitive diagnostic criteria include intraluminal filling defects and cutoff arteries as direct evidence of a thrombus (Figure 4). Other indirect signs such as hypoperfusion areas and asymmetric blood flow may be suggestive of PE but are not reliable when cardiorespiratory diseases coexist. PA is quite a safe examination, with mortality as low as 0.2%. However, this procedure has several limitations in clinical practice because it is invasive, expensive, and requires skillful physicians. It is also often unavailable in smaller hospitals. Relative contraindications include significant bleeding risk and renal insufficiency. Notably, a negative PA result does not fully exclude VTE. The 3-month VTE rate after a normal PA has been reported to be 1.7%. Furthermore, it has been suggested that PA has a limited interobserver agreement at subsegmental level (ranging between 45% and 66%) and that sensitivity for subsegmental emboli may be suboptimal. The use of conventional PA as an isolated diagnostic procedure has declined. PA has been abandoned in favor of noninvasive CTPA, which offers equivalent or even better information. Right ventriculography for the diagnosis of right ventricular failure from acute PE has been replaced by echocardiography and biomarkers. Furthermore, the risk of local bleeding complications increases if thrombolysis is attempted in patients in whom PE has been diagnosed by standard PA. PA should be performed as the final diagnostic test whenever a diagnostic dilemma persists after noninvasive imaging tests. Advantages of PA include the option of direct hemodynamic measurements and catheter-based interventions, such as local thrombolysis, mechanical clot fragmentation, or catheter embolectomy.

The advantage of PA is its high diagnostic accuracy. It allows direct hemodynamic measurements and offers the option of catheter-based treatments. However, the disadvantage of PA is that it is invasive, costly, requires considerable expertise, and is not widely available. Contraindications include significant bleeding risk and renal insufficiency.

**Echocardiography**

The contribution of echocardiography to the diagnosis of PE is most often indirect because direct visualization of emboli within the right atrium, right ventricle (RV), or pulmonary artery occurs occasionally in only 4% of the patients with acute PE. Indirect echocardiographic signs of PE predominantly include signs of right ventricular overload or dysfunction (Table 2, Figure 5). However, in a prospective study including unselected patients suspected of having massive PE, transthoracic echocardiography failed to identify 50% of patients with angiographically proven PE. Furthermore, echocardiographic signs of right ventricular overload or dysfunction are not specific and might be due to pre-existing cardiorespiratory diseases. There is evidence suggesting that some echocardiographic findings may be more specific. The 60/60 sign (acceleration time of RV ejection <60 ms and tricuspid insufficiency pressure gradient ≤60 mm Hg)

![Figure 4](image-url) Diagnosis of pulmonary embolism with invasive angiography. Selective invasive right pulmonary artery angiogram demonstrates occlusion of a side branch of the right inferior pulmonary artery (arrow) with consecutive missing vessels in the marked area (dotted circle). Pigtail catheter is visualized in the right main artery (asterisk).

**Table 2** Potential echocardiographic findings in patients with pulmonary embolism. A definite discrimination between acute and pre-existing chronic changes is not possible

<table>
<thead>
<tr>
<th>Condition</th>
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<tbody>
<tr>
<td>Right ventricular dilatation and hypokinesis</td>
</tr>
<tr>
<td>Paradoxical septal motion</td>
</tr>
<tr>
<td>Tricuspid regurgitation</td>
</tr>
<tr>
<td>Increased pulmonary artery pressure</td>
</tr>
<tr>
<td>Dilatation of inferior vena cava without inspiratorical collapse</td>
</tr>
<tr>
<td>Dilatation of proximal pulmonary arteries</td>
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Figure 5 Echocardiography in PE. A) Color Doppler imaging reveals large tricuspid valve regurgitation. B) 2D imaging with enlarged right ventricle with deviation of the interventricular septum to left (arrow). C) Doppler echocardiography is used for the assessment of the acute elevated pulmonary artery pressure. D) Dysfunction of the right ventricle reflected by a reduced TAPSE.

Abbreviations: 2D, two-dimensional; PE, pulmonary embolism; TAPSE, tricuspid annular plane systolic excursion; RA, right atrium; RV, right ventricle; LV, left ventricle.

and the McConnell sign (normal apical segment of the right ventricular free wall in combination with akinesia of the right ventricular mid-free wall) have been reported to be insensitive (25% and 19%), but highly specific (100% and 94%) for the diagnosis of acute PE.\textsuperscript{56} Consequently, systematic use of echocardiography for diagnosis in hemodynamically stable, normotensive patients is not recommended in the current European Guidelines (evidence level IIIC).\textsuperscript{57,58}

The main role of echocardiography in these patients is risk stratification since right ventricular dysfunction has been established as a powerful independent predictor of death from PE.\textsuperscript{52,59,60} Acutely unstable patients pose a different situation.
In these patients, the absence of right ventricular overload or dysfunction practically excludes PE and may provide information that helps in the differential diagnosis of other causes of shock including acute left ventricular dysfunction, tamponade, acute valvular disease, and aortic dissection. On the other hand, signs of right ventricular overload in a hemodynamically unstable patient with suspected PE are highly suggestive of PE and may justify thrombolysis if other diagnostic tests would result in additional risk or in delay of treatment. In support of this strategy, patients with a high clinical probability, a positive shock index (≥1), and presence of right ventricular dysfunction underwent such a treatment with an acceptable all-cause mortality rate of 5% at 30 days.

Because of the high prevalence of central PE in patients with hemodynamically significant PE, transesophageal echocardiography may allow direct visualization of a thrombus in the pulmonary artery and confirm the diagnosis in most cases. In a prospective study including 49 patients suspected of having massive PE with abnormal transthoracic echocardiograms, the sensitivity of transesophageal echocardiography for detecting PE of any size was 80% and its specificity was 100%.

In hemodynamically unstable patients, the advantage of bedside echocardiography is that it is a valuable alternative if CT is not immediately available and may guide treatment or help in the differential diagnosis of the cause of the shock. However, in hemodynamically stable patients, the disadvantage of echocardiographic signs of PE are their nonspecificity which may be due to concomitant cardiorespiratory diseases.

**Diagnostic algorithm**

The diagnostic strategy depends on the severity of PE, which is understood as a PE-related early mortality risk and allows a distinction between high-risk PE presenting with shock or hypotension and nonhigh-risk PE without shock or hypotension. This classification guides the choice of the optimal initial management strategy with the purpose to avoid unnecessary radiological exposure without losing a high sensitivity to exclude clinically significant PE.

In suspected nonhigh-risk PE, the diagnostic workup should include the triage of clinical decision rule, D-dimer testing, and, if necessary, imaging. The most frequently used clinical prediction rule is the Wells score which is a simple rule based on easily collected information (Table 1). It has been validated extensively using both a three-category (low, moderate, or high clinical probability) and a two-category scheme (PE likely or unlikely).

Several management studies have shown that PE can be ruled out without the need for further imaging in patients with low clinical probability and a normal D-dimer. Imaging is necessary in patients who either have an abnormal D-dimer result or have a high probability of PE irrespective of the D-dimer result. The first-line imaging modality is CTPA. CT-based algorithms have been well validated in prospective trials to safely diagnose or rule out PE. Clinicians should consider additional imaging whenever CTPA is inconsistent with the clinical probability.

For patients with contraindication to CTPA, V/Q scan is a valid option. In the current guidelines for V/Q scintigraphy, V/Q-SPECT is recommended as the procedure of choice and is preferred to V/Q planar (evidence level IIb). A normal V/Q-SPECT practically excludes PE, and positive findings lead to treatment in nearly all cases. Additional testing is required whenever lung scans are nondiagnostic. If the clinical suspicion of PE persists despite a negative V/Q planar result, the diagnosis should be rigorously pursued. PA should be reserved for patients in whom a high clinical suspicion of PE persists despite a normal or noninvasive imaging.

The diagnostic approach to suspected high-risk PE is different. Patients with high-risk PE presenting with hypotension or shock generally have a high clinical pretest probability. The most useful test in this situation is bedside transthoracic echocardiography, which will show indirect evidence of acute right ventricular overload or dysfunction if acute PE is the cause of hemodynamic condition. Rarely, right heart thrombi can be found on transthoracic echocardiography as a direct sign. In highly unstable patients or if other tests are not immediately available, the bedside echocardiographic findings alone may establish the diagnosis of PE. If the patient is stabilized, a definitive diagnosis should be sought by CTPA, which is usually able to confirm the diagnosis because of the high thrombus load in the pulmonary circulation.

In pregnant women with the suspicion of PE, radiation exposure of the fetus is a concern. However, in most cases, the use of ionized radiation is indispensable as PE is a potentially fatal diagnosis. CTPA delivers a higher radiation dose to the mother, but a lower dose to the fetus than V/Q scanning. Thus, if necessary, CTPA is preferred during pregnancy in all trimesters.

A straightforward diagnostic algorithm for suspected PE is presented in Figures 6 and 7.
PE-related early mortality risk

High-risk PE

CTPA available?

No

Yes

CTPA

RV - overload?

Yes

PE diagnosed

PE excluded

No

Echocardiography

PE diagnosed

PE excluded

Nonhigh risk PE

Clinical probability assessment

Low/intermediate probability

High probability

D-dimer

PE diagnosed

PE excluded

CTPA

PE excluded

PE distinguished according to PE-related early mortality risk distinguishes high-risk PE (ie, presenting with shock or hypotension) and nonhigh-risk PE (ie, presenting without shock or hypotension). Clinical probability is most commonly assessed by Wells’ criteria.

Note: 1 When CTPA is contraindicated, V/Q scanning is an alternative.

Abbreviations: PE, pulmonary embolism; CTPA, computerized tomographic pulmonary angiography; RV, right ventricle; V/Q, ventilation-perfusion.

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Figure 6
Algorithm in PE diagnosis. Risk stratification according to PE-related early mortality risk distinguishes high-risk PE (ie, presenting with shock or hypotension) and nonhigh-risk PE (ie, presenting without shock or hypotension). Clinical probability is most commonly assessed by Wells’ criteria.

Abbreviations: PE, pulmonary embolism; CTPA, computerized tomographic pulmonary angiography; RV, right ventricle; V/Q, ventilation-perfusion.

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Figure 7
Algorithm for diagnostic imaging with V/Q scintigraphy. V/Q-SPECT is the procedure of choice. V/Q scans are interpreted according to the holistic principle in which clinical pretest probability is a part.

Abbreviations: V/IQ-SPECT, ventilation-perfusion single photon emission computed tomography; V/Q planar, planar ventilation/perfusion; CTPA, computerized tomographic pulmonary angiography; PE, pulmonary embolism.
Imaging techniques for pulmonary embolism

Conclusion
The routine application of imaging techniques is not warranted in patients with a low probability clinical assessment in combination with a negative D-dimer result. When imaging is necessary, CTPA is the first-line test for investigating suspected PE. V/Q scintigraphy remains a validated option but is less frequently performed due to the high proportion of inconclusive results in planar V/Q scans. PA has been the traditional gold standard for the diagnosis of PE, but it is now rarely performed. In skillful hands, PA can be used when confirmation is required after uncertain results of noninvasive imaging tests. Echocardiography has a limited clinical utility in the diagnosis of PE in hemodynamically stable, normotensive patients with suspected PE. Only in hemodynamically unstable patients is the detection or absence of echocardiographic signs of right ventricular dysfunction significant and justify initiation or withholding of thrombolytic therapy.

Despite the limited sensitivity and specificity of individual imaging techniques, their combination with the clinical assessment and D-dimer testing is a key step in all diagnostic algorithms and provides a useful strategy for risk stratification and optimal treatment in patients with suspected PE.

Disclosure
The authors report no conflicts of interest in this work.

References


