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Dentinogenesis Imperfecta

Evaluation of Superior Vena

Highlights

The Role of Multislice CT

The Investigation of Bacteria

Discovering Thoughts, Inventing Future

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USA Toll Free Fax: +001-888-839-7392

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The Role of Multislice CT in Evaluation of Superior Vena Cava Syndrome

By Youssriah Yahia Sabri, Marian Fayek Farid, Sara M saleh & Hebatallah Assal

Cairo University Hospitals, Egypt

Abstract- The superior vena cava (SVC) syndrome is a clinical entity caused by obstruction of the superior vena cava by infiltration, compression or thrombosis. Cancer is the most common underlying cause of superior vena cava obstruction. The incidence of catheter-induced superior vena cava obstruction is rapidly increasing. Fibrosing mediastinitis and Behçet disease are rare causes of SVC syndrome. Clinical presentation of SVC syndrome may include cough, dyspnea, dysphagia, and swelling or discoloration of the neck, face and upper extremities. *Aim of this study* is to evaluate the role of Multislice CT in study of superior vena cava obstruction syndromes and assessment of collateral circulation in different causes of superior vena caval obstruction.

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The Role of Multislice CT in Evaluation of Superior Vena Cava Syndrome

Youssriah Yahia Sabri [α], Marian Fayek Farid [σ], Sara M saleh [ρ] & Hebatallah Assal [ω]

Abstract- The superior vena cava (SVC) syndrome is a clinical entity caused by obstruction of the superior vena cava by infiltration, compression or thrombosis. Cancer is the most common underlying cause of superior vena cava obstruction. The incidence of catheter-induced superior vena cava obstruction is rapidly increasing. Fibrosing mediastinitis and Behçet disease are rare causes of SVC syndrome. Clinical presentation of SVC syndrome may include cough, dyspnea, dysphagia, and swelling or discoloration of the neck, face and upper extremities. *Aim of this study* is to evaluate the role of Multislice CT in study of superior vena cava obstruction syndromes and assessment of collateral circulation in different causes of superior vena caval obstruction.

I. Introduction

The superior vena cava (SVC) syndrome is a clinical entity caused by obstruction of the superior vena cava by infiltration, compression or thrombosis. Although clinical symptoms of the disorder were first described in 1757 in a patient with a syphilitic aneurysm of the ascending aorta, vascular causes are now rare and approximately 90% of cases are associated with a cancerous tumor that is compressing the superior vena cava, such as bronchogenic carcinoma including small cell and non-small cell lung carcinoma, Burkitt's lymphoma, lymphoblastic lymphomas, acute lymphoblastic leukemia (rare), and other acute leukemias *(Krimsky et al, 2002)*. Tuberculosis has also been known to cause superior vena cava syndrome (SVCS). SVCS can be caused by invasion or compression by a pathological process or by thrombosis in the vein itself, although this latter is less common (approximately 35% due to the use of intravascular devices) *(1).*

Malignant superior vena cava obstruction In most published studies, cancer is the most common underlying cause of superior vena cava obstruction e.g. lung cancer and lymphoma *(Sakura et al, 2007)*. Most primary malignant tumors of the superior vena cava, such as leiomyosarcoma or angiosarcoma, are un-common. The presence of arterial enhancement of the thrombus is highly suggestive of tumor thrombus *(2).*

Author α σ: MD, Radiology Department, Thoracic Imaging Unit, Cairo University, Cairo, Egypt. e-mail: marian.fayek@hotmail.com
Author ρ: MSc, Radiology Department, Thoracic Imaging Unit, Cairo University, Cairo, Egypt.
Author ω: MD, Chest department, Kasr Al-Aini, Cairo University, Cairo, Egypt.

Benign Causes of Superior Vena Cava Obstruction *Iatrogenic superior vena cava obstruction*— the incidence of catheter-induced superior vena cava obstruction is rapidly increasing e.g. large central venous catheters, such as dialysis catheters, Hickman catheters, and parenteral nutrition catheters *(3). Fibrosing mediastinitis* is a rare histologically benign disorder caused by proliferation of collagen tissue and fibrosis in the mediastinum *(4). Behçet disease* is a rare systemic disease in which superior vena cava stenosis or occlusion may result *(5).*

Clinical presentation of SVC syndrome: SVC syndrome is caused by gradual compression of the SVC, leading to edema and retrograde flow, but it can also be caused more abruptly in thrombotic cases. Symptoms may include cough, dyspnea, dysphagia, and swelling or discoloration of the neck, face and upper extremities. Often, collateral venous circulation causes distension of the superficial veins in the chest wall. Although SVC syndrome is usually a clinical diagnosis, plain radiography, computed tomography (CT) and venography are used for confirmation. (6).

CT Findings: CT can diagnose SVC affection in SVC syndrome and can detect subclinical superior vena cava obstruction in patients who are relatively asymptomatic *(Yu JB et al, 2008).* Regardless of its cause, the CT diagnosis of superior vena cava obstruction includes lack of opacification of the superior vena cava, an intraluminal filling defect or severe narrowing of the superior vena cava, and visualization of collateral vascular channels *(Eren et al, 2006).* MDCT, with its multiplanar and 3D imaging capabilities, allows thorough anatomic delineation of the various collateral pathways diverting the blood from the site of obstruction. Although axial images allow evaluation of potential causes of superior vena cava obstruction, such as a mediastinal mass, MDCT provides information about the level and degree of superior vena cava obstruction, the length of the affected segment, and the presence or absence of intraluminal clot distal to the obstruction, thereby allowing the interventional radiologist to choose the optimal treatment option. *(Plekker et al, 2008).*

II. Aim of the Work

To evaluate the role of Multislice CT in study of superior vena cava obstruction syndromes and

assessment of collateral circulation in different causes of superior vena caval obstruction.

III. Patients and Methods

a) Patients

This study involved 20 patients; 13 males and 7 females, age range 15-69 (average of 39.3years). Cases were referred from the chest department to the radiology department in Kasr Al-Aini for MSCT of the chest.

Twelve patients presented clinically with mediastinal syndrome; of whom six cases were biopsy proved central Bronchogenic carcinoma, five were known cases of Behçet disease and one case with unknown etiology.

Two cases had right upper limb DVT. Two cases had lymphoma and one case was surveyed for chest metastases. One case had fever of unknown origin after chemotherapy. One case with anti-phospholipid antibody syndrome where echo detected calcified right atrial thrombus. One case with bilateral pleural effusion. (See table 1).

Table 1 : Showing patients' presentation

Referred case	Number of patients
Mediastinal syndrome	12
Upper limb DVT	2
Chest assessment in case of lymphoma	2
Chest assessment for metastases	1
Fever of unknown origin after chemotherapy	1
Antiphospholipid antibody syndrome	1
Bilateral pleural effusion	1

b) Methods

All patients were subjected to:

1. Thorough clinical examination with history taking, general and chest examination.
2. Routine laboratory tests mostly complete blood picture, other tests were considered according to case e.g. culture and sensitivity.
3. MSCT of the chest: Toshiba Aquilion MSCT 64 channels was used. All cases were given pump IV administration of 40 ml of omnipaque 350 mg/ml at a rate of 3 ml/sec.

IV. Results

See the summary of MSCT results in tables 2-4. Table 2 shows summary of the MSCT findings in our 20 cases, while table 3 gives summary of the SVC different MSCT appearances and table 4 shows collateral circulation in MSCT.

Table 2 : Summary of MSCT Findings

Case presentation	MSCT finding
5 Behçet cases	-Partial SVC obstruction -Eminent **mediastinal** collaterals
6 central infiltrating Bronchogenic carcinoma	Partially Infiltrated SVC with few collaterals noted in 5 cases and eminent **chest wall** collaterals in one case (the case was a follow up case of Bronchogenic carcinoma after radiotherapy).
Case of mediastinal syndrome of unknown etiology	Diffuse affection of the mediastinum with soft tissue density lesion causing effacement of SVC and marked attenuation of the right pulmonary artery ; a picture suggesting fibrosing mediastinitis* Eminent **chest wall** collaterals seen.
2 cases with left upper limb DVT	DVT seen extending into left brachiocephalic vein. Normal contralateral brachiocephalic vein and SVC.
2 cases with lymphoma	Huge anterior mediastinal mass .Displaced and compressed SVC. No collaterals
One case of chest assessment for metastases	Lung, pleura and mediastinal metastases .SVC distortion and partial infiltration . No collaterals
One case of fever of unknown origin after chemotherapy	Partial SVC obstruction with a heterogenous thrombus**with air density, enlarged SVC, no collaterals
One case of Antiphospholipid antibody syndrome	Partial SVC obstruction at lowest portion , no collaterals
One case of bilateral pleural effusion	Partial SVC thrombus in relation to CV-Line catheter, no collaterals

*case was proved fibrosing mediastinitis.

** Case was proved to have MRSA infection.

Table 3 : Summary of MSCT SVC findings

MSCT SVC findings	No. of cases
Partial obstruction	9 cases
Infiltration	7 cases
Normal	4 cases

Table 4 : MSCT detection of collaterals

No of cases	HRCT finding
5 Behçet cases	Eminent collaterals
1 case of fibrosing mediastinitis	Eminent collaterals
1 case of infected thrombus	No collaterals
2 cases with partial thrombus	No collaterals
5 cases of malignant mediastinal infiltration by Bronchogenic carcinoma	Few collaterals mostly chest
1 case of follow up Bronchogenic carcinoma with SVC infiltration	Eminent chest wall collaterals
1 case of 2ry malignant mediastinal infiltration.	No collaterals

V. Discussion

Multi–detector row computed tomography (CT) with 2D and 3D reconstructed images provides a unique perspective on thoracic anatomy and disease. Multi–detector row CT allows shorter acquisition times, greater coverage, and superior image resolution *(7).*

In vascular imaging, this would provide image quality that equals or surpasses that of conventional angiography. Its use has expanded to aid in diagnosis and surgical planning, as it is reliable in depicting clot, thoracic vasculature and may also be used to evaluate thoracic venous anomalies and to plan therapy *(8).*

Multi–detector row CT has been used for venous angiography. Superior vena cava obstruction, often related to tumor, is frequently seen at multi–detector row CT. This technique may be used to establish the extent of tumor involvement and document the extent of collateral vessel formation *(9).*

MDCT, with its multi-planar imaging capabilities, allows thorough anatomic delineation of the various collateral pathways diverting the blood from the site of obstruction. Although axial images allow evaluation of potential causes of superior vena cava obstruction, such as a mediastinal mass, if intervention is warranted, MDCT provides valuable information about the level and egree of superior vena cava obstruction, the length of the affected segment, and the presence or absence of intra-luminal clot distal to the obstruction, thereby allowing the interventional radiologist to choose the optimal treatment option *(10).*

Superior vena cava syndrome usually presents more gradually with an increase in symptoms over time as malignancies increase in size or invasiveness *(11).* The severity of the syndrome depends on the rapidity of onset of the obstruction and its location *(12).* (see table 5).

Table 5 : Proposed grading system for superior vena cava syndrome: (Wilson, 2007).The blue one

Grade	Category	Incidence %	Definition
0	Asymptomatic	10	Radiographic superior vena cava obstruction in the absence of symptoms
1	Mild	25	Edema in head or neck (vascular distention), cyanosis, plethora
2	Moderate	50	Edema in head or neck with functional impairment (mild dysphagia, cough, mild or moderate impairment of head, jaw or eyelid movements, visual disturbances caused by ocular edema)
3	Severe	10	Mild or moderate cerebral edema (headache, dizziness) or mild/moderate laryngeal edema or diminished cardiac reserve (syncope after bending)
4	Life-threatening	5	Significant cerebral edema (confusion, obtundation) or significant laryngeal edema (stridor) or significant hemodynamic compromise (syncope, hypotension, renal insufficiency)
5	Fatal	<1	Death

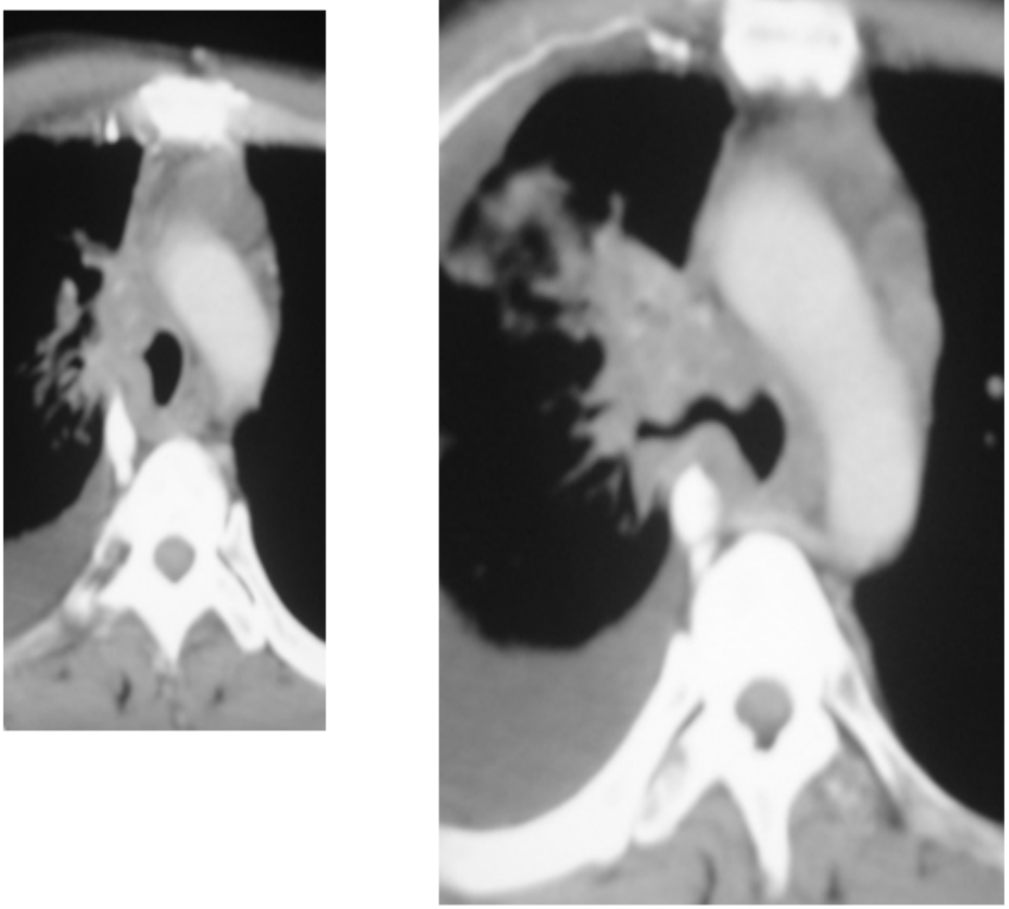
SVC syndrome can lead to the formation of downhill esophageal varices and pleural effusion. Numerous case reports have described pleural effusions in conjunction with the SVC syndrome. These effusions occur in 60% of SVC syndrome cases. The effusions are small, usually occupying less than one-half of the affected hemithorax, and occur approximately equally on either side or bilaterally. Although previously thought to be largely transudates, a large case series found that 18% of the effusions were chylous, with the remainder being exudates. None of the effusions sampled in the series were transudates. Occluded lymphatic flow from increased hydrostatic pressure in the SVC and left brachiocephalic vein probably contributes to the development of chylous pleural fluid. The pathophysiology of the exudative effusions, however, remains unknown. Many factors, including diuresis, small pulmonary emboli, and the underlying inflammatory or malignant condition all likely contribute. Chylous or exudative pleural effusions occur in most patients with SVC syndrome. The effusions are usually small and resolve upon correction of the underlying SVC obstruction. ***(13).***

Five to 10 percent of cases of SVC obstruction are due to benign causes. Most result from invasive monitoring techniques, such as the placement of central venous lines, Swan-Ganz catheters, and interventional techniques, such as the placement of pacemakers and central venous catheters for chemotherapy ***(14).***

The most common malignant causes are non–small-cell lung cancer (fig 1,2). Other types of cancer that can lead to this condition include: Breast cancer, lymphoma, metastatic lung cancer (lung cancer that spreads), testicular cancer, thyroid cancer, thymic tumors. (fig 3) ***(15).***

Case 1

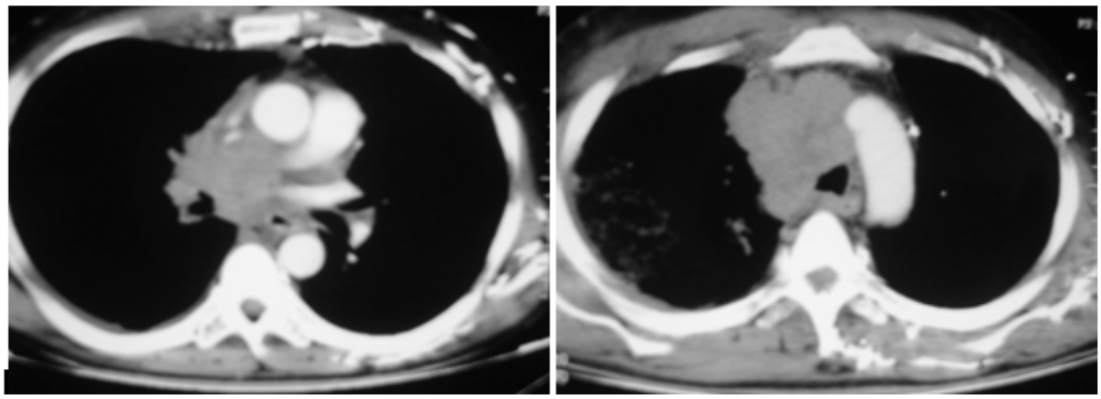
- Clinical Data
  48 year-old male patient known central Bronchogenic carcinoma presenting with mediastinal syndrome.
- MSCT



CECT chest mediastinal window axial images at different levels showing right central bronchogenic carcinoma with partial infiltration of SVC and chest wall collaterals. Note prominent azygos vein.

Case 2

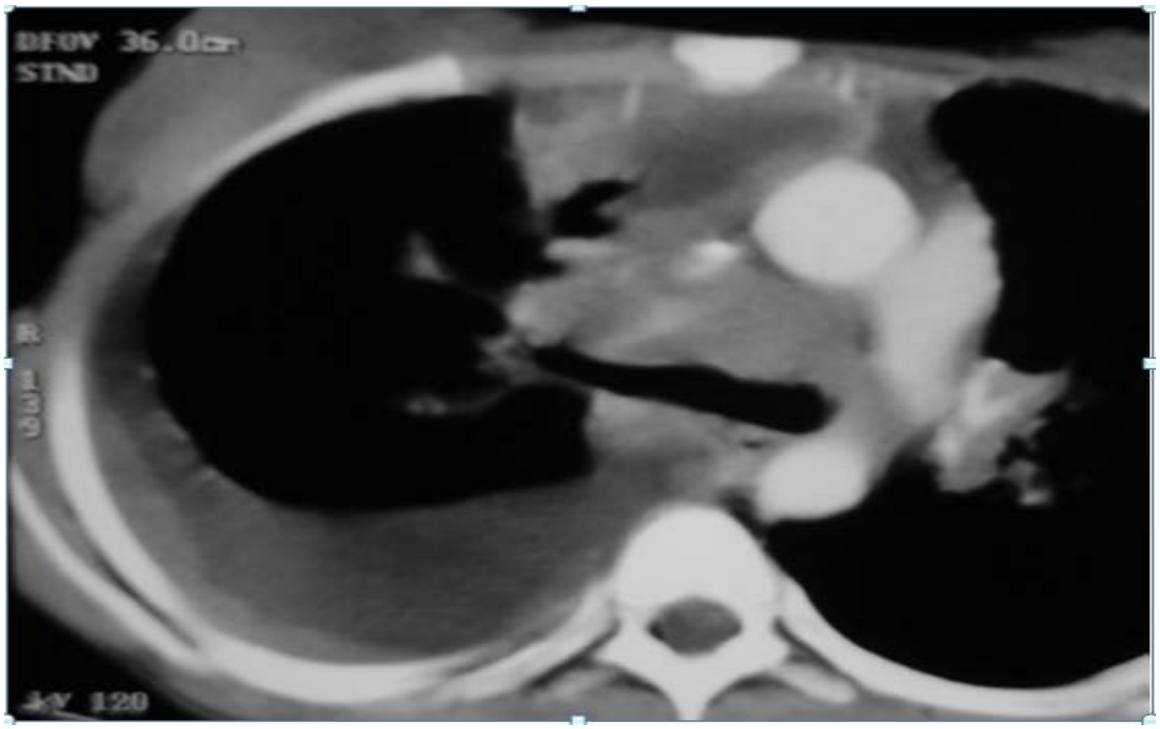
- **Clinical Data**
  39 year-old male patient known central Bronchogenic carcinoma with mediastinal syndrome. Case coming for follow up after radiotherapy.
- **MSCT**



CECT chest mediastinal window axial images at different levels showing right central bronchogenic carcinoma with partial infiltration of SVC and chest wall collaterals. Note non-enhanced azygos vein.

Case 3

- **Clinical Data**
  15 year-old female patient known ovarian carcinoma surveyed for metastases.
- **MSCT**



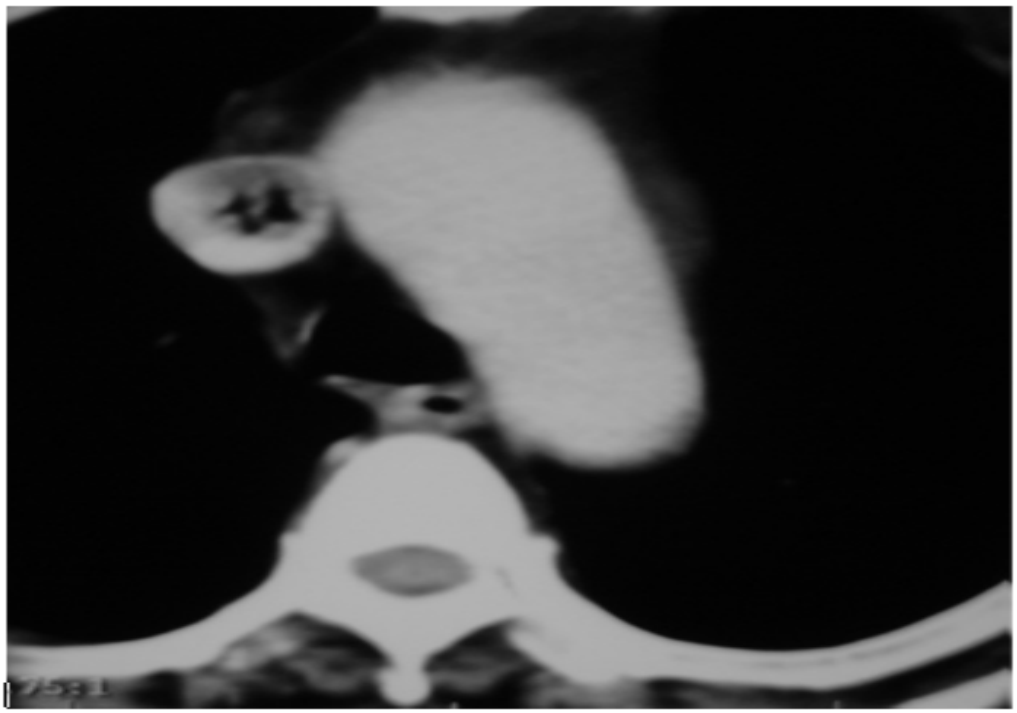
CECT **chest** mediastinal window axial image showing right moderate pleural effusion with thickened enhanced parietal pleura and mediastinal infiltration causing distortion and infiltration of SVC. No collaterals detected.

Non malignant conditions causing superior vena cava syndrome (SVCS) include mediastinal fibrosis; vascular diseases such as aortic aneurysm, vasculitis, and arteriovenous fistulas; infections such as histoplasmosis, tuberculosis, syphilis, and actinomycosis; benign mediastinal tumors such as teratoma, cystic hygroma, thymoma, and dermoid cyst; cardiac causes, such as pericarditis and atrial myxoma; and thrombosis related to the presence of central vein catheters. These account for approximately 22% of the causes of superior vena cava syndrome (SVCS) ***(16).***

- Iatrogenic superior vena cava obstruction (fig 4)—Iatrogenic causes of SVCS include venous thrombosis as a consequence of central venous catheters or pacemaker catheters and also fibrosis, caused by radiation therapy of the mediastinum.
- Thrombi in the SVC were detected by trans-esophageal echocardiography in 30% of patients who had single lumen silicone rubber hemo-dialysis catheters. Polyvinyl chloride, polyethylene and teflon catheters are associated with increased thrombogenisity, as compared to silicone rubber. Furthermore, the SVC stenosis may be induced by persistent trauma of the endothelium by the catheter tip and the higher blood flow during dialysis hours *(17).*
- Fibrosing mediastinitis (fig 5)—it is a rare benign disorder caused by proliferation of acellular collagen and fibrous tissue within the mediastinum. Although many cases are idiopathic, many (and perhaps most) cases in the United States are thought to be caused by an abnormal immunologic response to Histoplasma capsulatum infection. Affected patients are typically young and present with signs and symptoms of obstruction or compression of the superior vena cava, pulmonary veins or arteries, central airways, or esophagus. There may be two types of fibrosing mediastinitis: focal and diffuse. The focal type usually manifests on computed tomographic (CT) or magnetic resonance (MR) images as a localized, calcified mass in the para-tracheal or sub-carinal regions of the mediastinum or in the pulmonary hila. The diffuse type manifests on CT or MR images as a diffusely infiltrating, often non-calcified mass that affects multiple mediastinal compartments. CT and MR imaging play a vital role in the diagnosis and management of fibrosing mediastinitis *(18).*
- Behçet disease (fig 6,7) —Behçet disease is a multisystem disease of unknown etiology. The syndrome carries the name of the Turkish dermatologist Hulusi Behçet, who, in 1937, described a syndrome of recurrent aphthous ulcers, genital ulcerations, and uveitis leading to blindness. Although the cause of the disease is still unknown, it has become recognized as a multisystemic inflammatory disease *(19).*
- Enlargement of the thyroid gland (goiter) *(20).*

Case 4

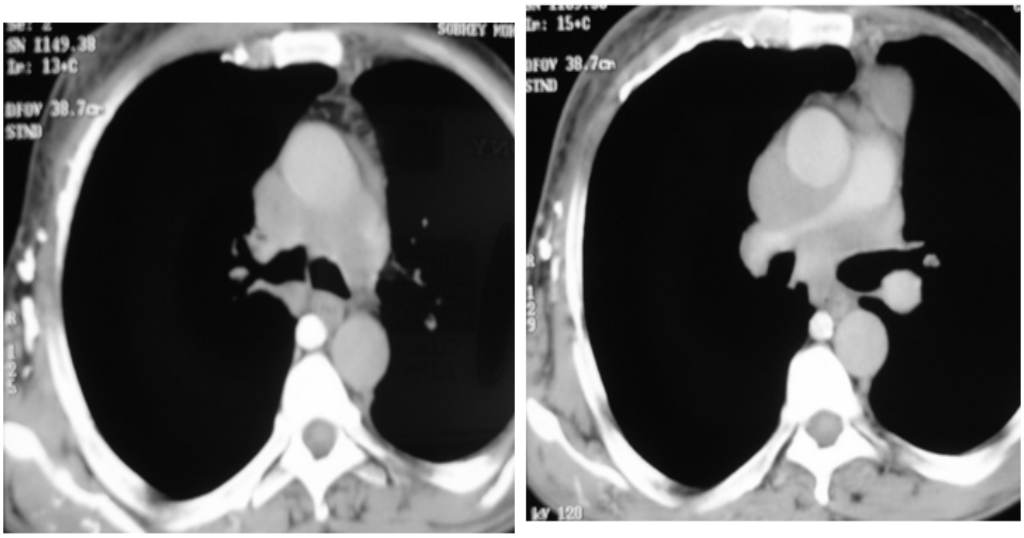
- Clinical Data
  60 year-old female patient investigated for fever of unknown origin.SVC heterogenous thrombus by CECT proved to be MRSA infected.
- MSCT



CECT chest axial image mediastinal window shows Partial SVC obstruction with a heterogeneous thrombus with air density, enlarged SVC, no collaterals.

Case 5

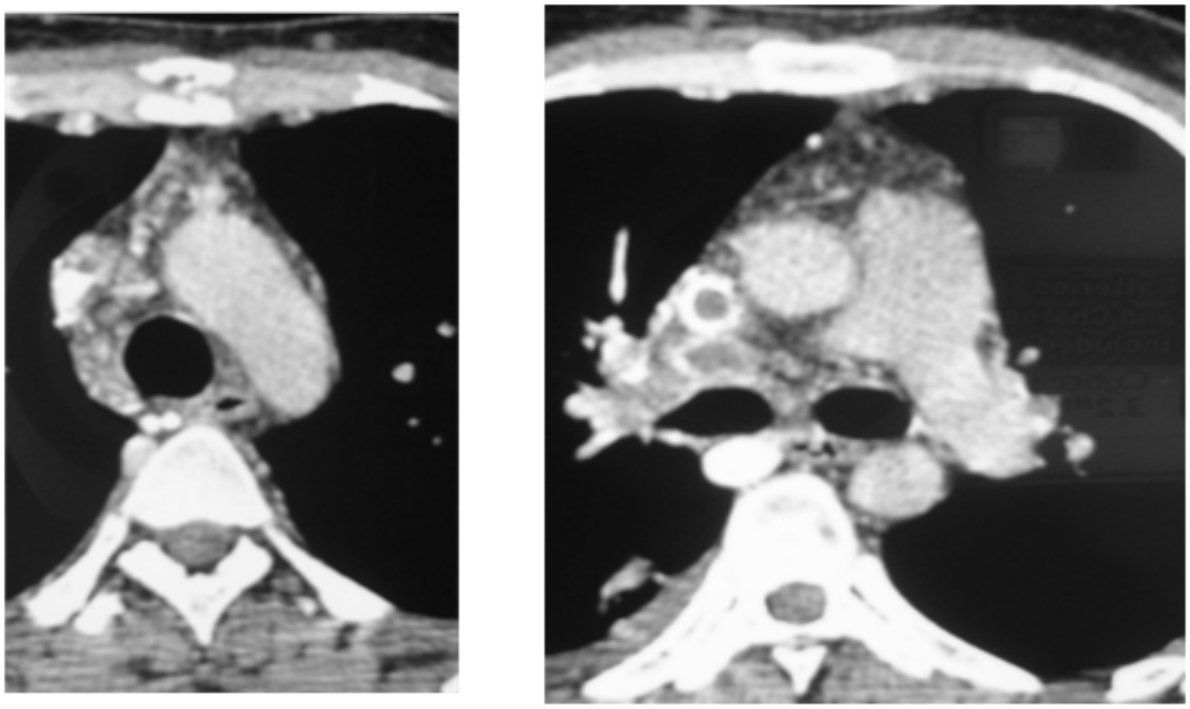
- **Clinical Data**
  65 year-old female patient with mediastinal syndrome proved to be fibrosing mediastinitis.
- **MSCT**



CECT chest mediastinal window axial images at different levels in a case of fibrosing mediastinitis showing effacement of SVC and chest wall collaterals. Note prominent azygos vein. Marked attenuation of the right pulmonary artery.

Case 6

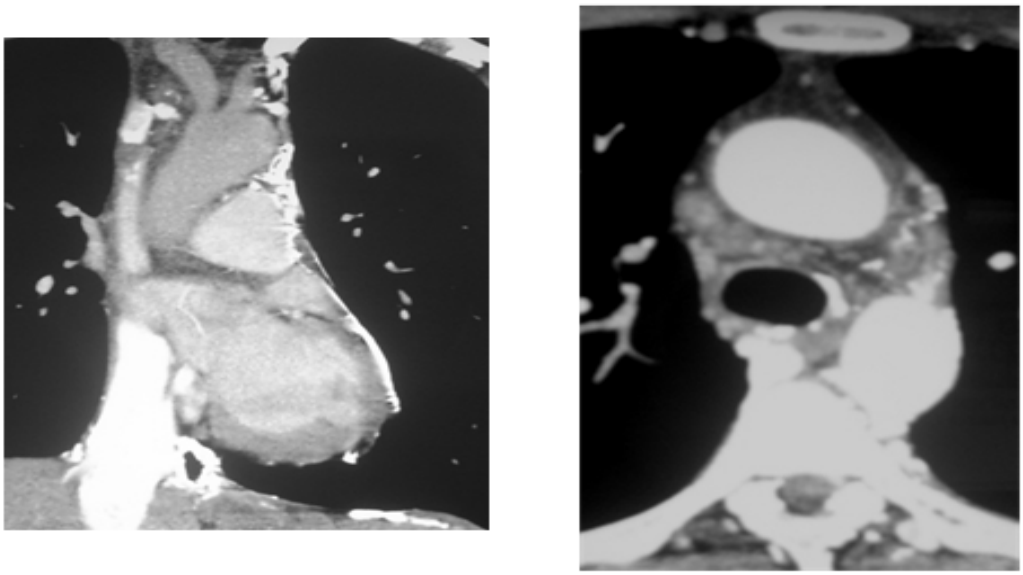
- **Clinical Data**
  28 year-old male patient known Behçet presenting with mediastinal syndrome.
- **MSCT**



CECT chest mediastinal window axial images at different levels showing partial thrombus of SVC with eminent mediastinal collaterals. Note prominent azygos vein.

Case 7

- **Clinical Data**
  31 year-old male patient known Behçet presenting with mediastinal syndrome.
- **MSCT**



CECT chest mediastinal window sagittal and axial images partial thrombus of SVC with eminent mediastinal collaterals. Note prominent azygos vein.

The aim of this study was to provide evidence that MSCT is useful in patients with superior vena caval obstruction, detecting the level and the cause of obstruction.

Multi-slice CT scans were reviewed in 20 patients referred from chest department to radiology department in Kasr Al-Aini.

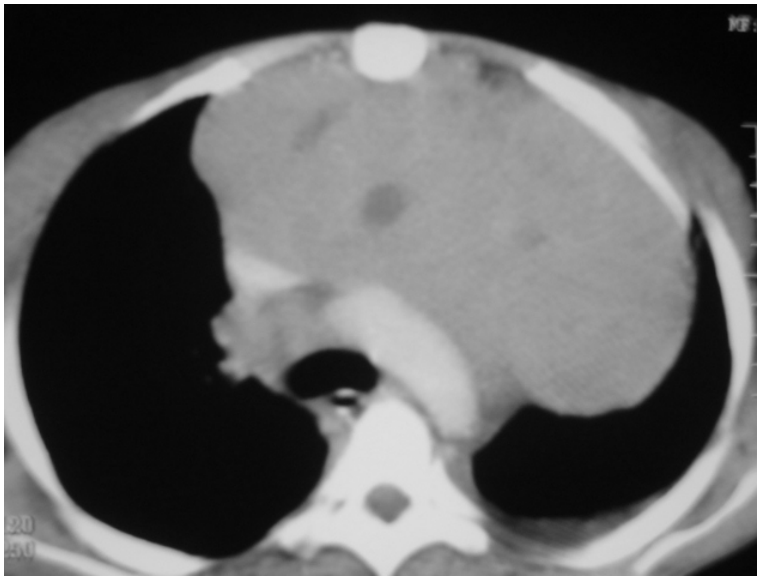
Twelve patients presented clinically in our study with mediastinal syndrome, Two cases had right upper limb DVT. Two cases had lymphoma and one case was surveyed for chest metastases. One case had fever of unknown origin after chemotherapy. One case with anti-phospholipid antibody syndrome where echo detected calcified right atrial thrombus. One case with bilateral pleural effusion

The twelve patients presented clinically in our study with mediastinal syndrome, six cases were biopsy proved central Bronchogenic carcinoma, five were known cases of Behçet disease and one case with unknown etiology. These findings are more or less consistent with the study made by Bagheri et al, 2009 which stated that among 45 patients with SVCS, their diagnostic pathological reports showed 26 (57.8%) were compatible with bronchogenic carcinoma (small cell lung cancer [SCLC] in 19 cases and non-SCLC in 7) which was the most common etiology of superior vena cava syndrome. Lymphoma was reported in 14 cases (31.1%), (12 of non-Hodjkin's lymphoma and 2 of Hodjkin's lymphoma), and germ cell tumor and malignant thymoma were observed in 3 (6.7%) and 2 (4.4%) of patients ***(21).***

Among the twenty cases reviewed in our study, there were 2 cases diagnosed to have lymphoma (fig 8); MSCT revealed huge anterior mediastinal mass, displaced and compressed SVC with no collaterals. These findings are not consistent with the study made by ***Kantarci et al, 2008*** which presented multi-detector row CT (16-detector scanner) features of a case of SVC syndrome caused by compression of lymphadeno-pathies at Hodgkin lymphoma of an 8-year-old girl. Her chest radiograph showed mediastinal enlargement and computed tomography of the chest showed massive mediastinal lymphadenopathy, axillary adenopathy, and bilateral pleural effusions. Multi Slice Computed tomography showed no contrast within the SVC and contrast within ***enlarged collateral venous channels*** of the left chest and no channels on the right because of the right subclavian venous occlusion ***(22).***

Case 8

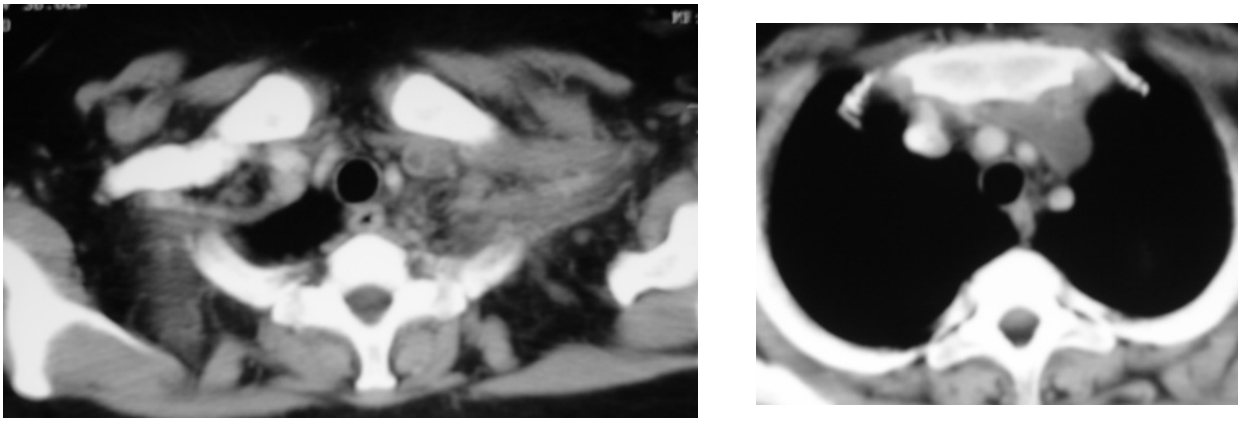
- Clinical Data
  17 year-old female patient known Hogkin's lymphoma.
- MSCT



CECT chest axial image mediastinal window showing huge anterior mediastinal mass compressing and posteriorly displacing SVC.

Case 9

- Clinical Data
  37 year-old female patient with left upper limb DVT.
- MSCT



CECT chest axial images mediastinal window showing the swollen non enhanced left brachiophephalic vein with normal right brachiocephalic vein and SVC.

Osman Temizöz et al, 2010 reported a 50-year-old female patient with BD who was admitted to hospital with a 4-week history of persistent dyspnea and chest pain. Physical examination revealed swelling of the neck and upper extremities. In addition, there were visible venous collateral channels, particularly on the right lateral side of her upper trunk. A clinical diagnosis of SVC syndrome was made, and MDCT of the chest and abdomen revealed occlusion of the SVC. The dilated right lateral chest wall veins were seen as collateral channels crossing the right diaphragm The azygos and hemiazygos veins were also dilated. These findings are more or less consistent with our study having 5 cases of Behcet's disease; their CT scans revealed eminent mediastinal collaterals with prominent azygos vein *(23).*

In our study, 5 Behçet cases revealed partial SVC obstruction and eminent mediastinal collaterals, 6 cases with central infiltrating Bronchogenic carcinoma revealed partially infiltrated SVC with few collaterals noted in 5 cases and eminent chest wall collaterals in

one case (the case was a follow up case of Bronchogenic carcinoma after radiotherapy), 1 Case of mediastinal syndrome of unknown etiology showed diffuse affection of the mediastinum with soft tissue density lesion causing effacement of SVC and marked attenuation of the right pulmonary artery ; a picture suggesting fibrosing mediastinitis with eminent chest wall collaterals, 2 cases with left upper limb DVT seen extending into left brachiocephalic vein and normal contra-lateral brachiocephalic vein and SVC, 2 cases with lymphoma showed huge anterior mediastinal mass, displaced and compressed SVC with no collaterals, one case of chest assessment for metastasis revealed lung, pleura and mediastinal metastases with SVC distortion and partial infiltration and no collaterals, one case of fever of unknown origin after chemotherapy showed partial SVC obstruction with a heterogenous thrombus with air density, enlarged SVC, no collaterals, one case of Antiphospholipid antibody syndrome revealed partial SVC obstruction at lowest portion , no collaterals, one case of bilateral pleural effusion revealed partial SVC thrombus in relation to CV-Line catheter, no collaterals.

To conclude, the rate of obstruction and its location greatly affects the severity of symptoms of SVCS, this depends on the development of the collateral circulation. Meaning that when the onset of obstruction is slow (as in benign causes of obstruction e.g. Behcet's disease and with central venous catheters), the collateral circulation will have time to distend and accommodate the increased blood flow. On the other hand, diseases causing rapid onset of obstruction (malignant tumors), produce more severe symptoms because the collateral veins will not have time to distend. *Longmore et al, 2007* suggested that the general recruitment of venous collaterals over time may lead to remission of the syndrome although the SVC remains obstructed *(24).*

Wilson, 2007 stated that not all cases of SVC obstruction must present with SVCS (e.g. facial edema, venous distension in the neck, upper limb edema) and that there is 10% of cases may have radiographic SVC obstruction in absence of symptoms. This is consistent with our study which includes twelve of the twenty patients presented clinically with mediastinal syndrome, two cases had right upper limb DVT, two cases had lymphoma and one case was surveyed for chest metastasis. One case had fever of unknown origin after chemotherapy, one case with anti-phospholipid antibody syndrome and one case with bilateral pleural effusion *(25).*

VI. Summary and Conclusion

Superior vena cave syndrome (SCVS) is a constellation of signs and symptoms resulting from obstruction of the SVC or its major tributaries by intra-luminal occlusion or by extrinsic compression and/or invasion from malignant and benign diseases.

SVC obstruction leads to increased venous pressure and edema of the neck, arms, upper chest, and head causing increased intracranial pressure. Patient may present with headache, syncope or pre-syncope, nausea and vomiting, hoarseness, dysphagia, cough, dyspnea and chest pain. Severity of symptoms depend on the time course of obstruction. As obstruction develops, venous collaterals develop to find alternate pathways for venous return to the right atrium. In the post-antibiotic era malignancy remains the commonest etiology. Lung cancer is the commonest malignancy. SVCS is most common with small cell lung cancer as it grows rapidly in central airways. CT chest is the investigation of choice which provides information on location, possible etiology, extent of collaterals and guide biopsy attempts.

The advent of multi-detector CT has revolutionized imaging of the mediastinal vascular structures. In comparison to single-detector helical CT scanners, multi-detector scanners not only provide faster speed, greater coverage, and improved spatial resolution, but also have the unique ability to create images of thick and thin collimation from the same data set.

One of the greatest benefits of this new technology is the improved quality of two-dimensional (2D) multi-planar and three-dimensional (3D) reconstruction images.

MSCT can easily prove or exclude the affection of SVC by partial or complete obstruction, the development of collateral circulation as well as detecting the cause of obstruction whether thrombosis, compression or infiltration and its extent.

Reference Références Referencias

1. Ansari MJ, Syed A, Wongba W, et al.: Superior vena cava obstruction presenting as a complication of repeated central venous cannulations. Compr Ther. 2006;32:189–91.
2. Garlitski AC, Swingle JD, Aizer A, et al.: Percutaneous treatment of the superior vena cava syndrome via an excimer laser sheath in a patient with a single chamber atrial pacemaker. J Interv Card Electrophysiol. Sep 2006;16(3):203-6.
3. Rice TW, Rodriguez RM, Barnette R, et al: Prevalence and characteristics of pleural effusions in superior vena cava syndrome.Respirology 2006 ;11:299-305.
4. Yangui F, Battesti JP, Valeyre D et-al.: Fibrosing mediastinitis as a rare mechanism of pulmonary oedema in sarcoidosis. Eur. Respir. J. 2010;35 (2): 455-6.
5. Hiller N, Lieberman S, Chajek-Shaul T, et al: Thoracic manifestations of Behcet disease at CT. Radiographics 2004; 24:801–808.
6. Khalili B, Boggs PB, Bahna SL, et al: Superior vena cava syndrome: A masquerader of anaphylaxis. J Allergy Clin Immunol. 2007;119(Supple 1):S29.

7. Jonathan F.Wan, BASc,MD, FRCPC, Andrea Bezjak, MD, MSc, FRCPC: Superior Vena Cava Syndrome, Emerg Med Clin N Am 27 (2009) 243–255.
8. Abeloff, M., Armitage, J., Niederhuber, J., et al: Clinical Oncology "Superior vena cava syndrome: an oncologic complication "Seminars in Oncology Nursing" 2004. 12 (4):312-7.
9. Rubin GD, Shiau MC, Leung AN, et al: Aorta and iliac arteries: single versus multiple detector-row helical CT angiography. Radiology 2000; 215:670–676.
10. Rizvi AZ, Kalra M, Bjarnason H, et al: Benign superior vena cava syndrome: stenting is now the first line of treatment. J Vasc Surg 2008; 47:372–380
11. Michael S Beeson: MD, MBA, FACEP, Professor of Emergency Medicine, Northeastern Ohio Universities College of Medicine and Pharmacy; Attending Faculty, Summa Health System. Updated: Dec 3, 2009.
12. Longmore, Murray; Ian Wilkinson, et al: Superior Vena Cava Syndrome, Oxford Handbook of Clinical Medicine. Oxford. (2007). p. 162.
13. Kantarci, Mecit MD, PhD; Fil, Fadime MD; et al: Superior Vena Cava Syndrome in a Child and Venous Collateral Pathways: MDCT Imaging, Journal of Thoracic Oncology, Volume 3 - Issue 8, 2008; pp 915-916.
14. Weber T, Roth TC, Beshay M, et al.: Video-assisted thoracoscopic surgery ofmediastinal bronchogenic cysts in adults: a single-center experience. Ann Thorac Surg 78:987–991,2004.
15. Watkinson AF, Yeow TN, Fraser C.: Endovascular stenting to treat obstruction of the superior vena cava.BMJ. Jun 21 008;336(7658):1434-7.
16. Akoglu H, Yilmaz R, Peynircioglu B, et al.: A rare complication of hemodialysis catheters: superior vena cava syndrome. Hemodial Int. Oct 2007;11(4):385-91.
17. Oguzkurt L, Tercan F, Yildirim S, et al: Central venous stenosis in haemodialysis patients without a previous history of catheter placement. Eur J Radiol 2005; 55:237.
18. Santiago E. Rossi, M ,H. Page McAdams, MD, Melissa L. Rosado-de Christenson, et al: fibrosing mediastinitis. RadioGraphics 2001; 21:737-757.
19. Saadoun D, Wechsler B, Desseaux K, et al.: Mortality in Behçet's disease. Arthritis Rheum. Sep 2010;62(9):2806-12.
20. Ugras-Rey S, Watson M.: Selected oncologic emergencies. In: Marx JA, Hockberger RS, Walls RM, et al, eds. Rosen's Emergency Medicine: Concepts and Clinical Practice. 7th ed. Philadelphia, Pa: Mosby Elsevier;2009:chap 121.
21. Bagheri R, Rahim M, Rezeetalab F, et al: Malignant superior vena cava syndrome: is this medical emergency. Ann Thorac Cardiovasc surg, vol (15) no 2, 2009: 89-92.
22. Kantarci, Mecit MD, PhD; Fil, Fadime MD; et al: Superior Vena Cava Syndrome in a Child and Venous Collateral Pathways: MDCT imaging, Journal of Thoracic Oncology, Volume 3 - Issue 8, 2008; pp 915-916.
23. Osman Temizöz, Hakan Genchellac, Ensar Yekeler, et al: CT-angiographic demonstration of hepatic collateral pathways due to superior vena cava obstruction in Behçet disease, 2010.
24. Longmore, Murray; Ian Wilkinson, et al: Superior Vena Cava syndrome, Oxford Handbook of Clinical Medicine. Oxford. (2007). p. 162.
25. Wilson LD, Detterbeck FC, Yahalom J.:Superior vena cava syndrome with malignant causes. N Engl J Med. 2007;356:1862-1869.

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Electron Microscopy Furthers the Investigation of Bacteria-Nanoparticles Interactions Sub-Cellular Dynamics

By Lyubov V. Didenko, Natalia V. Shevlyagina, Roberta Curia, Alessandro Erega & Marziale Milani

Gamaleya Research Institute for Epidemiology and Microbiology, Russian Federation

Abstract- Electron microscopy is useful in studying the interactions between *S. aureus* and polyurethane nanoparticles as good models for bacteria-polymer relations. A valuable ensemble of investigation tools allows not only to understand the cellular dynamics, but provides information about nanoparticles delivery (to host cells and consequently to tissues and organs) as well.

Analysis of the electron images can bring better comprehension of processes such as adhesion, in response to the reciprocal attraction between nanoparticles and cells, and endocytosis. Understanding the course of nanoparticles, we can suppose the existence of reversible mechanisms (exocytosis), and clear up how bacteria-host cells interactions work.

Keywords: S. aureus, nanoparticles, electron microscopy, polyurethane, biodestruction, endocytosis, bacteria-host cell interaction, toxicology.

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Lyubov V. Didenko [α], Natalia V. Shevlyagina [σ], Roberta Curia [ρ], Alessandro Erega [ω] & Marziale Milani [¥]

Abstract- **Electron microscopy is useful in studying the interactions between *S. aureus* and polyurethane nanoparticles as good models for bacteria-polymer relations. A valuable ensemble of investigation tools allows not only to understand the cellular dynamics, but provides information about nanoparticles delivery (to host cells and consequently to tissues and organs) as well.**

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Electron microscopy gives helpful answers for the research in toxicology and raises new questions about the relations between bacteria and polymeric materials.

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I. Introduction

Electron microscopy is a powerful mean through which it is possible to gain information at a cellular and sub-cellular level. The elements brought out by electron microscopy are not only about morphology, but regard the cellular dynamics and the roles of several structures as well. Images obtained on a Transmission Electron Microscope (TEM) can give valuable details about the interactions that occur at a cellular scale. Samples prepared and fixed, according to different protocols and procedures, are observed with the TEM, in TEM or STEM (Scanning Transmission Electron Microscope) mode. During the analysis different data collection techniques such as Bright Field (BF) and Dark Field (DF) are applied, each technique highlighting different details of the same sample's area collection techniques such as Bright Field (BF) and Dark Field (DF) are applied, each technique highlighting different details of the same sample's area

After the acquisition of the images, the main purpose is to identify the distinct structures in an unambiguous and objective way, possibly automating the whole process. This could be obtained processing the micrographs with an image editing software, combining personal skills and software tools. It would mean being able to increase on a large scale the number of events examined in reasonable time, favouring the statistics which generally are complicated to get in transmission microscopy. Unfortunately this approach has severe limitations since most of the times the image background prevents the setting of parameters, key of an automatic recognition, so that all the work relies on personal abilities.

In this work we would like to show how electron microscopy is a valid tool to investigate the *in vitro* interactions between bacteria and polymeric materials (polyurethane).

S. aureus is a Gram positive bacterium, normally present in the oral cavity, able to operate biodestruction over polyurethane prostheses [Didenko *et al.*, 2012]. *In vitro* experiments show that the incubation of polyurethane with *S. aureus* results in the formation of a biofilm [Arciola *et al.*, 2012] and, in the last stage, in the biodestruction of the polymeric material [Didenko *et al.*, 2012; Howard, 2011; Zachinyaev *et al.*, 2009]. The starting point of biofilm formation is the bacterial attachment to the polyurethane surface [Ghannoum and O'Toole, 2004; Smirnova *et al.*, 2010; Park *et al.*, 1998]. It has been demonstrated that the rougher is the surface, the faster is the adhesion [Satriano *et al.*, 2006; Yoda *et al.*, 2014]. After a long incubation time (> 25 days) the whole surface of polyurethane (both rough and smooth) is covered with biofilm [Didenko *et al.*, 2012]. After the irreversible adhesion of microorganisms to the plastic material, bacteria start the production of a matrix of extracellular polymeric substance (EPS) [Flemming and Wingender, 2010]. The EPS is generally composed of extracellular DNA, proteins and polysaccharides, and its functions are to protect and hold together the bacterial cells and to

Author α σ: Gamaleya Research Institute for Epidemiology and Microbiology, Gamaleya ul. 18, 123098 Moscow, Russian Federation.
Author ρ ω ¥: Department of Biotechnology and Biosciences – University of Milano-Bicocca, Via Cozzi 53, 20125 Milan, Italy.
Author ρ ω: Department of Biotechnology and Biosciences – University of Milano-Bicocca, P.zza della Scienza 2, 20125 Milan, Italy.
e-mail: r.curia@campus.unimib.it

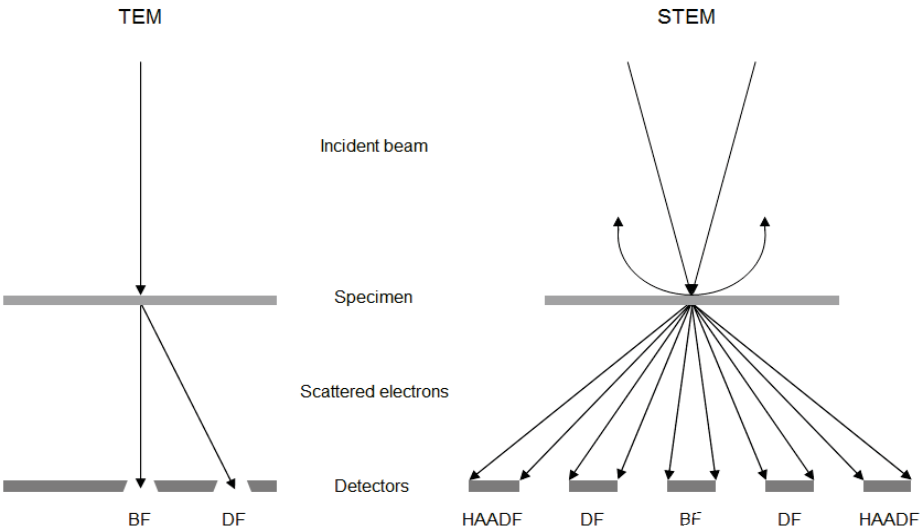

Figure 1 : The scheme shows how differently the incident beam works and how signals are collected in Transmission Electron Microscope (TEM) and Scanning Transmission Electron Microscope (STEM). When an electron beam hits a sample, electrons are scattered to different angles. Bright Field (BF), Dark Field (DF) and High Angle Annular Dark Field (HAADF) images are acquired collecting different signals

promote the communication among them [Bhattacharyya and Choudhury, 2008]. The following step is the maturation of the biofilm and the formation of microcolonies [Ghannoum and O'Toole, 2004; Smirnova *et al.*, 2010]. The number of bacterial cells increases, therefore there is a shortcoming of nutrients; moreover there is an increment of the environment acidity, due to the bacterial metabolic activity. Hence the polyurethane is weakened and bacteria attack the plastic material, already deteriorated by the low pH of the environment, in order to get some source of nourishment. The polyurethane degradation operated by *S. aureus* implies the detachment of little scraps of material that range from micrometers to nanometers. This has been demonstrated removing the biofilm from the polyurethane in a FIB/SEM (Focused Ion Beam/Scanning Electron Microscope) through ultrasounds and analyzing the polymeric surface [Didenko *et al.*, 2012]. It appears deeply modified, micro- or nano-patterned, and looks lacy.

Through electron microscopy we were able to investigate that *S. aureus* can internalize the nanosized debris of polyurethane (less than 10 nm). The internalization process of the polymeric material into bacterial cells occurs through endocytosis following a general scheme. In literature several types of internalization processes are discussed with different names, but globally they present the same general features [Doherty and McMahon, 2009; Iversen *et al.*, 2011]. Thanks to the electron images we collected shots of the several steps: approach of nanoparticles to the bacterial cell, formation of a vesicle for the absorption of nanoparticles and englobing of nanoparticles in vesicles inside the bacterial cell. Hence the issue of the toxicity of nanosized polyurethane raises [Revell, 2006; Gatti, 2004; Hoet *et al.*, 2004], in fact the same material in the bulk form is not toxic [Howard, 2011]. Electron images point out that, as a consequence of the endocytic process, polyurethane nanoparticles accumulate into bacterial cells.

With this work we would like to draw attention to the problem of the material toxicity, since it has been demonstrated the actual uptake and storage of polyurethane nanoparticles by *S. aureus*. Moreover, considering the possible dynamics of bacteria-host cells interactions, we can suggest mechanisms of nanoparticles spreading from bacteria to host cells and therefore to an entire organism.

II. Materials and Methods

Bacterial cells (*S. aureus*) were isolated from a patient suffering from a periodontal disease. A part of them was incubated in a nutrient broth as a control sample; the remaining part was incubated in a broth with polyurethane. The polymeric material, provided by Dentalur Russia, had different types of surfaces. The role of the polyurethane roughness is discussed in Satriano *et al.*, 2006 and in Yoda *et al.*, 2014. The control sample of the polymer was a smooth polyurethane slice in broth. Polyurethane and bacteria were incubated from 1 to 45 days at 37°C. Samples were prepared for the observation in TEM. Details of the preparation method can be found in Didenko *et al.*, 2012 and in Curia *et al.*, 2014.

TEM and STEM images were acquired on a FEI TECNAI F20 X-TWIN (FEI Company, USA) equipped with a 200 kV FEG column and a CCD detector. Using only an

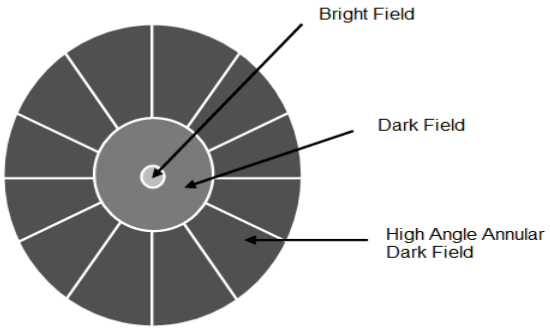

Figure 2 : Description of an annular CCD detector and its zones, as seen in the plane perpendicular to the incident beam

instrument for both the TEM and STEM modes, we were able to obtain two images of the very sample' spot, therefore we could analyze the TEM image and the STEM one, capturing the several details that the two modes bring out. Moreover the images were collected with two different techniques: Bright Field (BF) and High Angle Annular Dark Field (HAADF). These techniques differ from the way in which electrons hit the sample and build up the images (Fig. 1). In BF modality an aperture lets only the direct beam hit the sample, blocking the scattered electrons; as a result in BF images thin areas appear brighter than the thicker ones. When an electron beam hits an ultrathin sample, most of the electrons are scattered into high angles or backwards. In HAADF modality annular detectors (Fig. 2) collect the scattered electrons up to angles higher than 50 mrad. In HAADF images denser zones (objects) which have a higher atomic number Z and thus scatter stronger, appear bright, whereas thinner areas result darker than the thicker ones [Krumeich]. With these different techniques it is possible to obtain images with better contrast and resolution.

It is important to note that, under the same conditions of signal and detector, in STEM the resolution is a $\sqrt{2}$ factor better than in TEM. Moreover, as no lenses are used to form STEM DF images, they are less noisy than TEM DF ones [Utsunomiya and Ewing, 2003].

The images acquired have been processed with GIMP [available from http://www.gimp.org], an open source image editing software. Our aim was to locate nanoparticles and bacterial internal structures. We worked mostly balancing the contrasts and enhancing the edges, unfortunately, in our case GIMP did not resulted as efficient as a visual analysis, since the software was effective only in the identification of structures provided with large contours. Nanoparticles' edges detection cannot be faced with GIMP and we were able to identify only the membranous vesicles.

III. Results

Thanks to the electron images we were able to capture some of the events that occur in the bacterial cell and its surroundings in presence of polymers.

Fig. 3 is a TEM BF image of a cell of *S. aureus* after a long term *in vitro* incubation with polyurethane. It sums up the power of electron microscopy that, with only one image, can bring out a lot of information. In this image it is possible to observe polymeric nanoparticles (derived from biodestruction) out of the cell, on the cell wall and inside the cell, enclosed in vesicles. This shows which could be the possible course of nanoparticles from out the cell to inside the bacterium. In this shot it is also visible a ruffle, a possible step in the uptake process [Doherty and McMahon, 2009], in which are present vesicles loaded with nanoparticles. It is evident that vesicles can contain one or more nanoparticles. From the image we can deduce that not all the nanoparticles around the cell (whose size can be as large as 100 nm, as seen in Didenko *et al.*, 2013) enter *S. aureus*, but only those which measure less than 10 nm. They are enveloped in vesicles whose diameters and membrane are approximately 30 nm and 5 nm-thick, respectively.

Polyurethane nanoparticles have higher electron density than the cell biological components, so they appear darker than the surrounding medium in BF images (Figs. 3, 4, 6 and 7) and brighter in HAADF ones (Figs. 8 and 9) [Didenko *et al.*, 2013].

In Fig. 4 it is possible to single the vesicles out, spotting them both in the ruffle and in the cell. We obtained this images working with GIMP on the original one, balancing contrasts and enhancing the contours of the objects, within the bacterial cell, we were interested into (vesicles).

Looking at Figs. 3 and 4 in detail, one can clearly see that vesicles follow a line, as they were in a row driven by a supporting structure. In these images it is not evident what is carrying the vesicles, but the way in which they are disposed make us think that it is a cytoskeletal-like structure, probably a microtubule, whose existence and role are suggested in Amos *et al.*, 2004 and in Cabeen and Jacobs-Wagner, 2007. Moreover this points out the presence of a *continuum* of vesicles from the ruffle to the whole cell, strengthening the theses that the ruffle derives from an uptake process stimulated by the nanoparticles, and that the bacterial cytoskeleton is actually involved in the capture and internalization of the nanomaterial. The scheme of the vesicles' position is clarified in Fig. 5.

In the scheme displayed in Fig. 5 we have underlined the presence of the EPS matrix that has an important role in the approach of nanoparticles to the

cell, in the nanoparticles coating (protein corona), and in the internalization process, where the ruffle enters in connection with the matrix during endocytosis.

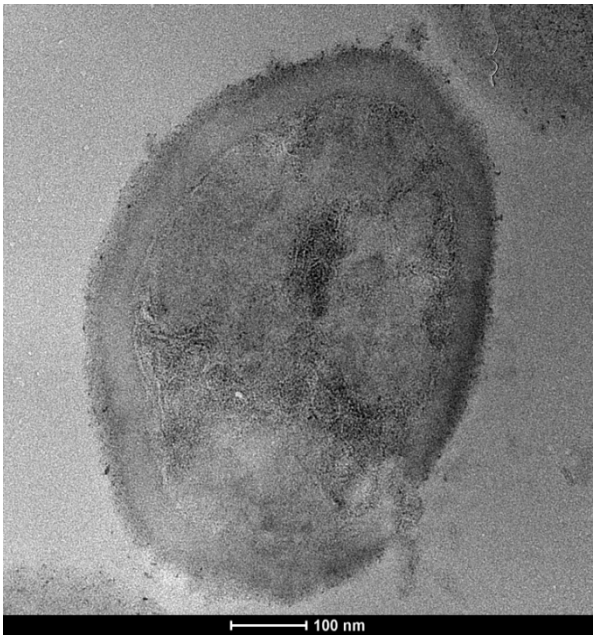

Figure 3 : TEM BF image of *S. aureus* after incubation with polyurethane. Polymeric nanoparticles can be found outside the cell, on the cell wall and inside the cell, surrounded by vesicles. Details of a ruffle and internal structures are present

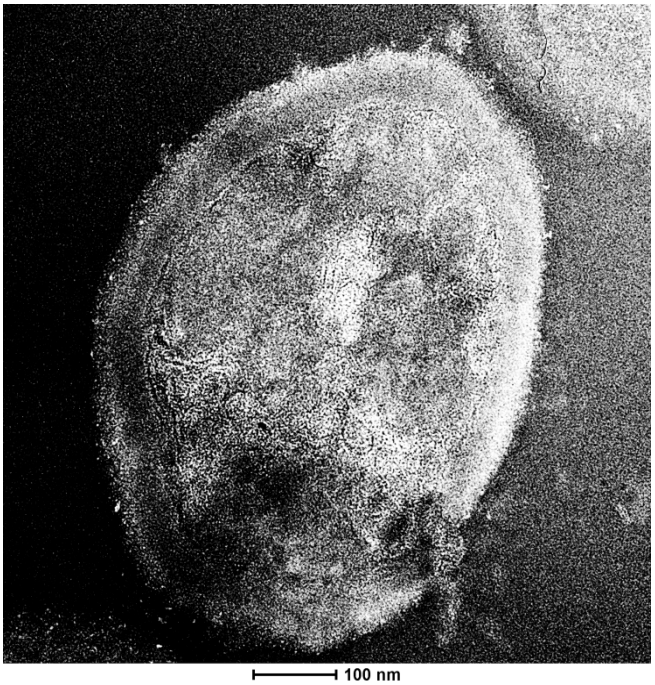

Figure 4 : Same image as in Fig. 3 rielaborated with GIMP in order to better display internal structures (vesicles)

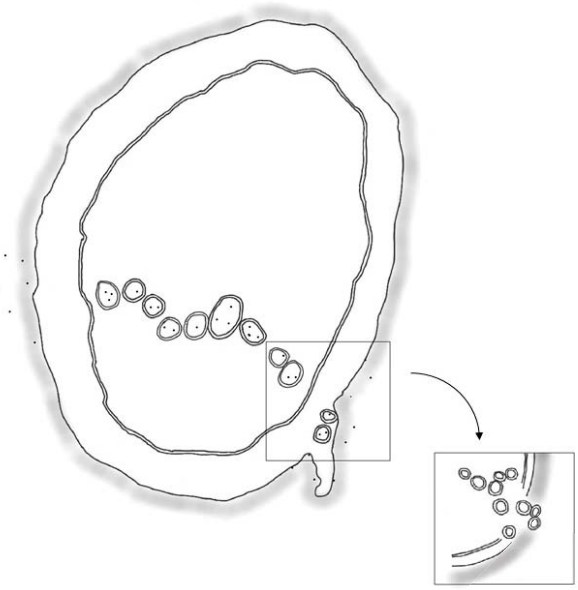

Figure 5 : Scheme of the spotting of some of the bacterial internal structures of interest according to different dimensional scales (nanoparticles, vesicles, vesicles' membrane, bacterial plasmatic membrane). This schematization, carried out through visual analysis and mixing of Figs. 3, 6 and 8 (the last two at a higher magnification), proves the existence of a *continuum* of vesicles from the ruffle to the whole cell

A portion of the same sample as in Fig. 3, with better magnification, is shown in Fig. 6 (TEM BF) and Fig. 8 (STEM HAADF). Fig. 6 gives a focus on the vesicles that go from the ruffle and across the cell, lying in a row; Fig. 8 offers a better possibility to observe the vesicles in the ruffle, showing in the meantime the clear contours of the vesicles inside the cell.

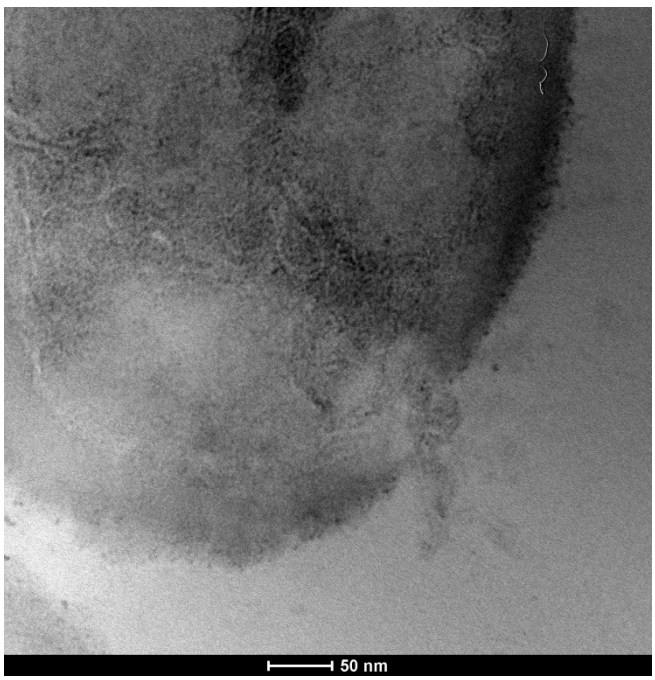

Figure 6 : TEM BF image of a portion of the same sample as in Fig. 3, with higher magnification. Details of a ruffle and nanoparticle loaded vesicles are present

Figs. 7 and 9 show our attempts to improve the visibility of internal structures with the use of GIMP. While in these images it is possible to identify the improved edges of some of the vesicles present, we can state that the result as a whole is not satisfying. In fact in this case the tools of GIMP do not allow to improve the identification of the internal structures, whereas a visual inspection is more efficient.

The limits of the use of editing image software are proved not only in Figs. 7 and 9 where it is impossible to clearly identify all the bacterial structures of interest, but in all of the other images as well, since GIMP allowed to focus only on objects provided with large contours (vesicles) and not on nanoparticles where edge detection is not feasible.

The above mentioned limits prove that in our research an automatic process for structures' recognition is far from reach.

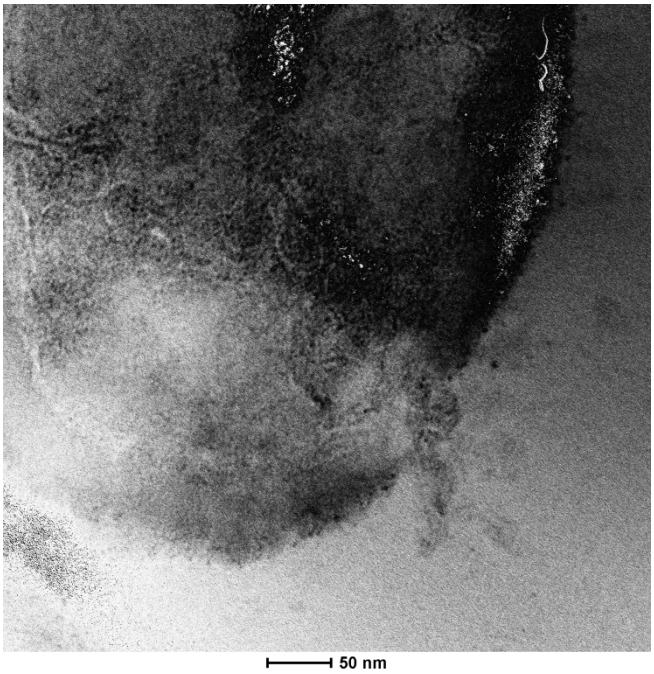

Figure 7 : Same image as in Fig. 6 rielaborated with GIMP in order to better display internal vesicles

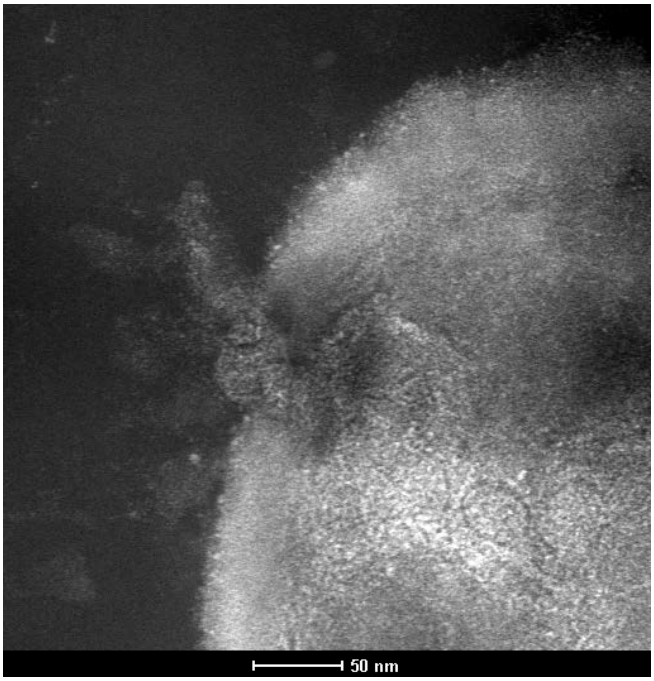

Figure 8 : STEM HAADF image of a portion of the same sample as in Fig. 3, with higher magnification. Details of a ruffle and nanoparticle loaded vesicles are present

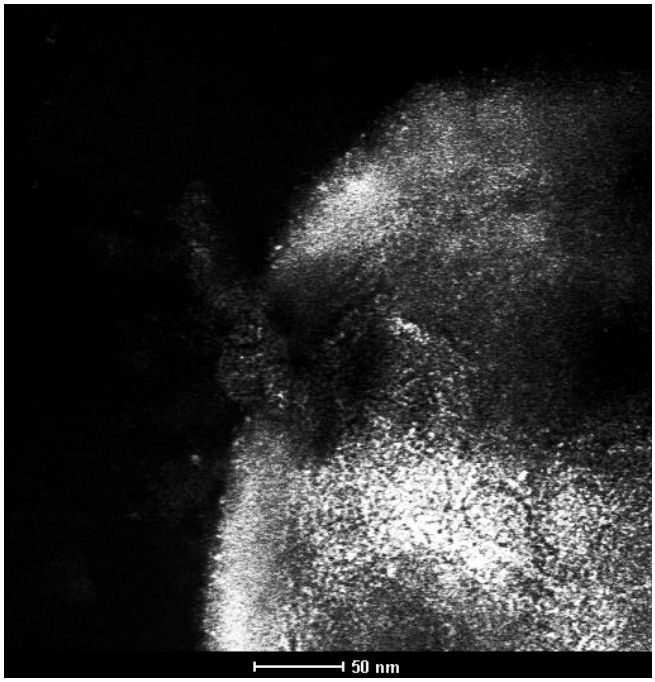

Figure 9 : Same image as in Fig. 8 rielaborated with GIMP in order to better display internal vesicles

IV. Discussion

The images in the previous section prove the importance of electron microscopy. Electron images suggest the possible endocytic pathway that nanoparticles take from outside the bacterium to the interior of the cell. Fig. 3 is shot in a fixed time and helps us to understand the space-time cellular dynamics. It is notable that from a single image we get information about the whole course of the polymeric material. The first step is the approaching of nanosized particles to the bacterial cell. Polyurethane nanoparticles can be positively or negatively charged, as well as neutral [Urquhart *et al.*, 1995; Watanabe *et al.*, 2003]. In all cases, the proximity of nanoparticles to the bacterial membrane brings into play electromagnetic forces. In fact, when approached by charged or neutral nanoparticles, the membrane high electric field (up to 10GV/m) interacts with permanent nanoparticle dipole moments (if present) and/or induces electrical dipoles [Korobeynikov *et al.*, 2002; Pekker and Shneider, 2014; Fröhlich, 1975; Davydov, 1982; Del Giudice *et al.*, 1985; Del Giudice *et al.*, 1986; Askar'yan, 1962; Ho *et al.*, 1994]. Moreover nanoparticles can be free or enveloped by a protein corona (not visible in our images). The corona is a protein cover, probably derived from the EPS matrix, which encloses nanoparticles [Lynch *et al.*, 2009; Lundqvist *et al.*, 2008; Walczyk *et al.*, 2010; Wang *et al.*, 2013]. Thus the protein corona, while covering its inner load to the bacterial cell, modifies the electromagnetic parameters of the nanoparticles-bacteria system. The role of the EPS matrix, consequently, is not limited to holding together and protecting bacterial cells, but it plays a fundamental role in the approach of nanoparticles to the cell and in the nanoparticles coating (protein corona).

The following step is the uptake of nanoparticles from *S. aureus*. This process occurs through endocytosis [Doherty and McMahon, 2009; Iversen *et al.*, 2011; Jermy, 2010] and starts with the folding of the plasma membrane: the bacterial membrane ruffles and the cell alters its connections with the EPS matrix. Subsequent events consist in the formation of membranous vesicles that surround the polymeric material and if present the protein corona as well, and in the actual internalization of the polyurethane into the bacterial cell. The action of the cytoskeleton components backs the whole endocytic pathway [Skruzny *et al.*, 2012] from the ruffling of the membrane to the formation and absorption of vesicles. *S. aureus* is able to englobe the foreign nano-material as a whole, incorporating it into vesicles. The existence of a bacterial cytoskeleton [Amos *et al.*, 2004; Cabeen and Jacobs-Wagner, 2007] and its important role are supported and underlined by electron images that show vesicles laying in a row, on a linear structure that could be a microtubule. Therefore electron microscopy illustrates not only the dynamics of the internalization processes, but provides information useful in the understanding of the highly controversial issues of bacterial cytoskeleton–like structures and functions [Amos *et al.*, 2004; Cabeen and Jacobs-Wagner, 2007], trying to clear up how they are involved in endocytosis.

The absorption of nanoparticles by bacterial cells (Fig. 10) studied *in vitro* suggests that similar events could occur *in vivo* as well.

S. aureus is a pathogen agent capable of reaching almost all the body districts, having various

targets such as tissues and organs, and able to provoke infections. Its pathogenicity is even worse when it is associated with resistance to antibiotics [Lowy, 2000; Malani, 2014; Sansonetti, 1993].

The uptake of nanoparticles by *S. aureus* has implications in the toxicological field [Curia *et al.*, 2013]. First of all because the nanoparticles we are taking into consideration are not engineered [Kettiger *et al.*, 2013; Simkó and Mattsson, 2010], in fact we are dealing with nanoparticles derived from the bacterial action against polymers (in our case polyurethane dental prostheses). It has already been assessed that bulk polyurethane is not toxic [Howard, 2011], but it is known that the properties of the same material change when its size approaches the nanoscale. The higher surface/volume ratio of nanoparticles compared to that of bigger particles, makes nanoparticles more readily absorbable by a cell [Revell, 2006; Gatti, 2004; Hoet *et al.*, 2004]. Moreover the uptake of nanoparticles does not affect the bacterial viability, so that *S. aureus* continues its course undisturbed [Didenko *et al.*, 2012; Didenko *et al.*, 2013; Curia *et al.*, 2013; Curia *et al.*, 2014].

Bacteria loaded with nanoparticles, while attacking the host cells (Fig. 11) of an organism of an organisms following their natural path [Lowy, 2000; Malani, 2014; Sansonetti, 1993], carry nanosized particles to numerous zones of the body, acting as Trojan Horses. Indeed they hide nanoparticles and, once reached the target, deliver nanosized polyurethane adding a

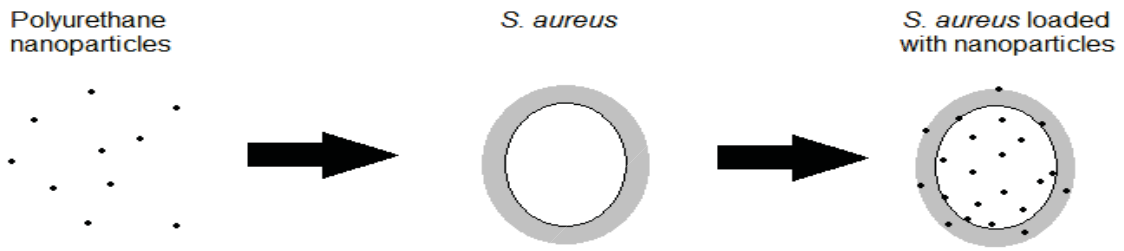

Figure 10 : Scheme of the absorption of polyurethane nanoparticles by *S. aureus*

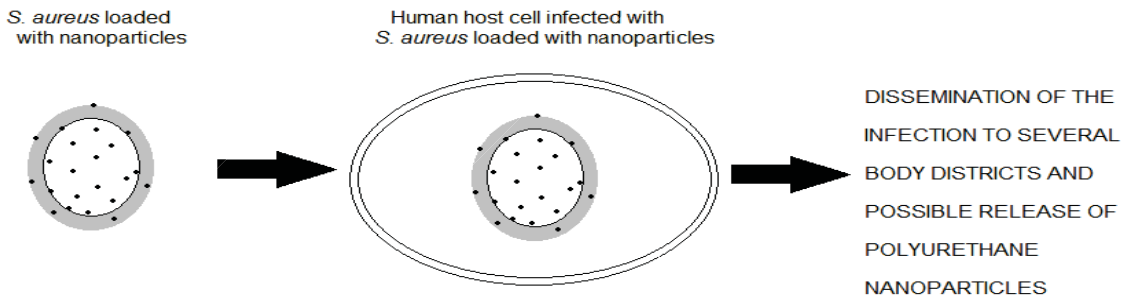

Figure 11 : Representation of the threat that bacteria loaded with nanoparticles stand for an human being

toxicological threat to the infection process [Curia *et al.*, 2013]. *S. aureus*, when in the host cell, can release nanoparticles that adhered to its outer membrane (nanoparticles with or without a protein corona, not enclosed in vesicles), can free nanoparticles without vesicles through exocytosis, or it can die loosening all its internal structures, vesicles filled with nanoparticles included [Curia *et al.*, 2013, Curia *et al.*, 2014]. The question of the existence of exocytic processes is under debate, as some researchers affirm that export processes are absent while others assume that internalization can be a reversible process [Dombu *et al.*, 2010; Salvati *et al.*, 2011].

The dissemination of nanoparticles implies that, due to the infection spreading, numerous organs (even far from one another and not directly exposed to the nanoparticles contamination) are invaded by nanoparticles [Teterycz *et al.*, 2010]. Moreover, nanoparticles released in the body by the bacteria-host cells chain (Fig. 11) can aggregate [Kasemets *et al.*, 2013] and the possible toxic material could reach high local concentrations, while the average concentration values are below the threshold levels. In the light of this, we suggest that the dosimetry levels of nanoparticles need to be rediscussed.

V. Conclusion

This work illustrates explains how much electron microscopy can contribute in the research of the consequences of biodestruction of medical prostheses operated by bacteria and in the resultant spreading of nanoparticles.

Electron images not only show bacterial internal structures and outline how they are involved in cellular dynamics, but prove the actual existence of nanoparticles uptake processes as well. All these information help to suggest unexplored paths about the nanoparticles delivery resulting from the interplay between *S. aureus* and host cells.

Being polyurethane a material commonly used in medical devices, it is of primary importance to deeply understand the mechanisms of the bacteria-host cells interactions during infectious processes, a threat always associated with implants and common to different bacteria and fungi [Teterycz *et al.*, 2010]. While electron microscopy manages to answer a few questions clearing up some of the points, it raises important issues about the dissemination of nanoparticles to organs not directly exposed to the menace, and about the potential toxicological concentration that nanoparticles can reach locally, at cellular level, bringing dosimetry up for discussion.

References Références Referencias

1. Didenko, L. V., Avtandilov, G. A., Shevlyagina, N. V., Smirnova, T. A., Lebedenko, I. Y., Tatti, F., Savoia, C., Evans, G. and Milani, M. (2012) "Biodestruction of polyurethane by *Staphylococcus aureus* (an investigation by SEM, TEM and FIB)", in Méndez-Vilas, A. (ed.) Current Microscopy Contributions to Advances in Science and Technology, Formatex Research Center Publishing, pp. 323-334.
2. Arciola, C. R., Campoccia, D., Speziale, P., Montanaro, L. and Costerton, J. W. (2012) " Biofilm formation in *Staphylococcus* implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials ", Biomaterials, vol. 33, pp. 5967-5982.
3. Howard, G. T. (2011) "Microbial biodegradation of polyurethane" in Fainleib, A. and Grigoryeva, O. (eds.) Recent Developments in Polymer Recycling, Transworld Research Network Publishing, Kerala, India, pp. 215-238.
4. Zachinyaev, Ya. V., Miroshnichenko, I. I. and Zachinyaeva, A. V. (2009) "Microbial degradation of PU", Russian Journal of Applied Chemistry, vol. 82, pp. 1321-1323.
5. Ghannoum, M. A. and O'Toole, G. A. (eds.) (2004) "Microbial Biofilms", Amer. Society for Microbiology Press, Washington DC, USA.
6. Smirnova, T. A., Didenko, L. V., Azizbekyan, R. R. and Romanova, Yu. M. (2010) "Structural and Functional Characteristics of Bacterial Biofilms", Microbiology, vol. 79, pp. 413-423.
7. Park, K. D., Kim, Y. S., Han, D. K., Kim, Y. H., Lee, E. H., Suh, H. and Choi, K. S. (1998) "Bacterial adhesion on PEG modified polyurethane surfaces", Biomaterials, vol. 19, pp. 851-859.
8. Satriano, C., Messina, G. M. L., Carnazza, S., Guglielmino, S. and Marletta, G. (2006) "Bacterial adhesion onto nanopatterned polymer surfaces", Materials Science and Engineering, vol. 26, pp. 942-946.
9. Yoda, I., Koseki, H., Tomita, M., Shida, T., Horiuchi, H., Sakoda, H. and Osaki, M. (2014) " Effect of surface roughness of biomaterials on *Staphylococcus epidermidis* adhesion ", BMC Microbiology, vol. 14, p. 234.
10. Flemming, H. C. and Wingender, J. (2010) "The biofilm matrix", Nature Reviews Microbiology, vol. 8, pp. 623-633.
11. Bhattacharyya, I. and Choudhury, M. (2008) "Quorum Sensing - Let Bacteria Talk", Advanced Biotech., vol. 7, pp. 30-33.
12. Doherty, G. J. and McMahon, H. T. (2009) "Mechanisms of Endocytosis", Annu. Rev. Biochem., vol. 78, pp. 857-902.
13. Iversen, T. G., Skotland, T. and Sandvig, K. (2011) "Endocytosis and intracellular transport of nanoparticles: present knowledge and need for future studies", Nano Today, vol. 6, pp. 176-185.
14. Revell, P. A. (2006) "The biological effects of nanoparticles", Nanotechnology Perceptions, vol. 2, pp. 283-298.
15. Gatti, A. M. (2004) "Nanopathology - The role of micro- and nano-particles in biomaterial-induced pathology", A RTD PROJECT QLRT-2001-147 (2002-2005) funded by European Commission.
16. Hoet, P. H. M., Nemmar, A. and Nemery, B. (2004) "Health impact of nanomaterials", Nature Biotechnology, vol. 22, p. 19.
17. Curia, R., Milani, M., Didenko, L. V., Avtandilov G. A., Shevlyagina, N. V. and Smirnova, T. A. (2014) "Beyond the biodestruction of polyurethane: *S. aureus* uptake of nanoparticles is a challenge for toxicology" in Méndez-Vilas. A. (ed.) Microscopy: advances in scientific research and education, Formatex Research Center Publishing, pp. 16-23.
18. Krumeich, F. " Properties of electrons, their interactions with matter and applications in electron microscopy",availablefrom:http://www.microscopy.ethz.ch/downloads/Interactions.pdf.
19. Utsunomiya, S. and Ewing, R. C. (2003) "Application of high-angle annular dark field scanning transmission electron microscopy, scanning transmission electron microscopy-energy dispersive X-ray spectrometry, and energy-filtered transmission electron microscopy to the characterization of

nanoparticles in the environment", Environ. Sci. Technol., vol. 37, pp. 786-791.
20. Didenko, L. V., Avtandilov, G. A., Shevlyagina, N. V., Shustrova, N. M., Smirnova, T. A., Lebedenko, I. Y., Curia, R., Savoia, C., Tatti, F., Milani, M. (2013) "Nanoparticles Production and Inclusion in *S. aureus* Incubated with Polyurethane: An Electron Microscopy Analysis", Open Journal of Medical Imaging, vol. 3, pp. 69-73.
21. Amos, L. A., van den Ent, F. and Löwe, J. (2004) "Structural/functional homology between the bacterial and eukaryotic cytoskeletons", Current Opinion in Cell Biology, vol. 16, pp. 24-31.
22. Cabeen, M. T. and Jacobs-Wagner, C. (2007) "Skin and bones: the bacterial cytoskeleton, cell wall, and cell morphogenesis", The Journal of Cell Biology, vol. 179, pp. 381-387.
23. Urquhart, S. G., Hitchcock, A. P., Leapman, R. D., Priester, R. D. and Rightor, E. G. (1995) "Analysis of polyurethanes using core excitation spectroscopy. Part I: Model polyurethane foam polymers", Journal of Polymer Science Part B: Polymer Physics, vol. 33, pp. 1593-1602.
24. Watanabe, M., Wakimoto, N., Shirai, H. and Hirai, T. (2003) "Bending electrostriction and space-charge distribution in polyurethane films", Journal of Applied Physics, vol. 94, pp. 2494-2497.
25. Korobeynikov, S. M., Melekhov, A. V., Furin, G. G., Charalambako, V. P. and Agoris, D. P. (2002) "Mechanism of surface charge creation due to image forces", J. Phys. D: Appl. Phys, vol. 35, pp. 1193-1196.
26. Pekker, M. and Shneider, M. N. (2014) "The surface charge of a cell lipid membrane", available from: http://arxiv.org/abs/1401.4707.
27. Fröhlich, H. (1975) "The extraordinary dielectric properties of biological materials and the action of enzymes", Proc. Natl. Acad. Sci. USA, vol. 72, pp. 4211-4215.
28. Davydov, A. S. (1982) "Biology and Quantum mechanics", Pergamon, Oxford.
29. Del Giudice, E., Doglia, S., Milani, M. and Vitiello, G. (1985) "A quantum field theoretical approach to the collective behaviour of biological systems", Nuclear Physics B, vol. 251, pp. 375-400.
30. Del Giudice, E., Doglia, S., Milani, M. and Vitiello, G. (1986) "Electromagnetic field and spontaneous symmetry breaking in biological matter", Nuclear Physics B, vol. 275, pp. 185-199.
31. Askar'yan, G. A. (1962) "Effect of the gradient of a strong electromagnetic beam on electrons and atoms", Sov. Phys. JETP, vol. 15, pp. 1088-1092.
32. Ho, M. W., Popp, F. A. and Warnke, U. (eds.) (1994) "Bioelectrodynamics and biocommunication", World Scientific Publishing.
33. Lynch, I., Salvati, A. and Dawson, K.A. (2009) "What does the cell see?", Nature Nanotechnology, vol. 4, pp. 546-547.
34. Lundqvist, M., Stigler, J., Elia, G., Lynch, I., Cedervall, T. and Dawson, K. A. (2008) "Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts", Proc. Natl. Acad. Sci. USA, vol. 105, pp. 14265-14270.
35. Walczyk, D., Baldelli Bombelli, F., Monopoli, M. P., Lynch, I. and Dawson, K. A. (2010) "What the cell "sees" in bionanoscience", J. Am. Chem. Soc., vol. 132, pp. 5761-5768.
36. Wang, F., Yu, L., Monopoli, M. P., Sandin, P., Mahon, E., Salvati, A. and Dawson K. A. (2013) "The biomolecular corona is retained during nanoparticle uptake and protects the cells from the damage induced by cationic nanoparticles until degraded in the lysosomes", Nanomedicine: Nanotechnology, Biology and Medicine, vol. 9, pp. 1159-1168.
37. Skruzny, M., Brach, T., Ciuffa, R., Rybina, S., Wachsmuth, M. and Kaksonen, M. (2012) "Molecular basis for coupling the plasma membrane to the actin cytoskeleton during clathrin-mediated endocytosis", Proc. Natl. Acad. Sci. USA, vol. 109, pp. E2533-E2542.
38. Jermy, A. (2010) " Evolution: Bacterial endocytosis uncovered ", Nature Reviews Microbiology, vol. 8.
39. Lowy, F. D. (2000) " Is *Staphylococcus aureus* an intracellular pathogen?", Trends in Microbiology, vol. 8, pp. 341-343.
40. Malani, P. N. (2014) " National burden of invasive methicillin-resistant *Staphylococcus aureus* infection ", JAMA, vol. 311, pp. 1438-1439.
41. Sansonetti, P. J. (1993) "Bacterial pathogens, from adherence to invasion: comparative strategies", Med. Microbiol. Immunol., vol. 182, pp. 223-232.
42. Curia, R., Milani, M., Didenko, L. V. and Shevlyagina, N. V. (2013) "Electron microscopy broadens the horizons of toxicology: The role of nanoparticles vehiculated by bacteria", Current Topics in Toxicology, vol. 9, pp. 93-98.
43. Kettiger, H., Schipanski, A., Wick, P. and Huwyler, J. (2013) "Engineered nanomaterial uptake and tissue distribution: from cell to organism", International Journal of Nanomedicine, vol. 8, pp. 3255-3269.
44. Simkó, M. and Mattsson, M. (2010) "Risks from accidental exposures to engineered nanoparticles and neurological health effects: A critical review", Particle and Fibre Toxicology, vol. 7, p. 42.
45. Dombu, C. Y., Kroubi, M., Zibouche, R., Matran, R. and Betbeder, D. (2010) "Characterization of endocytosis and exocytosis of cationic nanoparticles in airway epithelium cells", Nanotechnology, vol. 21, p. 355102.

46. Salvati, A., Åberg, C., Dos Santos, T., Varela, J., Pinto, P., Lynch, I. and Dawson, K. A. (2011) "Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics", Nanomedicine: Nanotechnology, Biology and Medicine, vol. 7, pp. 818-826.
47. Teterycz, D., Ferry, T., Lew, D., Stern, R., Assal, M., Hoffmeyer, P., Bernard, L. and Uçkay, I. (2010) "Outcome of orthopedic implant infections due to different staphylococci", International Journal of Infectious Diseases, vol. 14, pp. e913–e918.
48. Kasemets, K., Suppi, S., Künnis-Beres, K. and Kahru, A. (2013) " Toxicity of CuO Nanoparticles to Yeast *Saccharomyces cerevisiae* BY4741 Wild-Type and Its Nine Isogenic Single-Gene Deletion Mutants ", Chem. Res. Toxicol., vol. 26, pp. 356-367.

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Dentinogenesis Imperfecta Type II-A Case Report with Review of Literature

By Dr. Priya Singhal, Dr. Sugandha Arya, Dr. Manoj Vengal, Dr. Maitrey Bhalodia, Dr. Neelkanth Patil & Dr. Abhishek Pati

Vyas Dental College & Hospital, India

Abstract- Dentinogenesis imperfecta is a developmental disorder affecting the structure of the teeth.Both deciduous and permanent dentition are affected. Deciduous teeth are most severely affected.Early diagnosis and treatment are therefore important to obtain a better prognosis since late intervention makes treatment more complex.

Keywords: dentinogenesis imperfecta type ii, structural anomaly of teeth dental anomaly, pulp obliteration.

GJMR-D Classification : NLMC Code: WU 220

Strictly as per the compliance and regulations of:

Dentinogenesis Imperfecta Type II–A Case Report with Review of Literature

Dr. Priya Singhal [α], Dr. Sugandha Arya [σ], Dr. Manoj Vengal [ρ], Dr. Maitrey Bhalodia [ω], Dr. Neelkant Patil [¥] & Dr Abhishek Pati [§]

Abstract- **Dentinogenesis imperfecta is a developmental disorder affecting the structure of the teeth.Both deciduous and permanent dentition are affected. Deciduous teeth are most severely affected.Early diagnosis and treatment are therefore important to obtain a better prognosis since late intervention makes treatment more complex.**

Keywords: dentinogenesis imperfecta type ii, structural anomaly of teeth dental anomaly, pulp obliteration.

Clinical Relevance

Dentinogenesis imperfecta causes esthetic as well as functional problems to the patients. Early diagnosis may prevent patients from these problems and provide a life without nutritional deficits and psychosocial distress.

I. Introduction

Dentinogenesis imperfecta (DI) is an autosomal dominant genetic condition characterised by abnormal dentin structure affecting either the primary or both the primary and secondary dentitions. Incidence of DI is 1 in 6000 to1 in 80001.Clinically teeth are opalescent with the color ranging from bluish-gray to brown to yellowish and exhibit pronounced attrition of incisal and occlusal edges. Radiographically, crowns are bulbous with cervical constrictions and the roots are short. The pulp chambers and root canals are usually obliterated due to dentin over production. Synonyms are- *Hereditary opalescent dentine, Capdepont teeth.* Here we report a case of 21 year old male presenting with DI type II with clinical and radiographic features.

II. Case Report

A 21 year old male visited to the Dept Of Oral Medicine and Radiology with the chief complaint of dull, diffuse pain in the maxillary teeth and poor aesthetics due to rapid wearing of the tooth surface. Oral examination revealed generalized opalescent teeth with yellowish brown discoloration. (Fig1a,1b.)Generalized chipping of enamel was present. Fractured cusps were present in multiple teeth.(Fig 2a,2b).No bone abnormality was present. Patient's medical history was non contributory. The family history suggested that the patients maternal grand-father and mother had the the same features. Orthopantomograph (OPG) and Full mouth radiographs (IOPAs) (fig 3a,3b) were taken as an investigation. Short roots, missing pulp chambers, obliteration of canals, multiple periapical radiolucencies were the striking features in the radiograph. Diagnosis of Dentinogenesis imperfecta typeII was given on the basis of clinical and radiographic features.

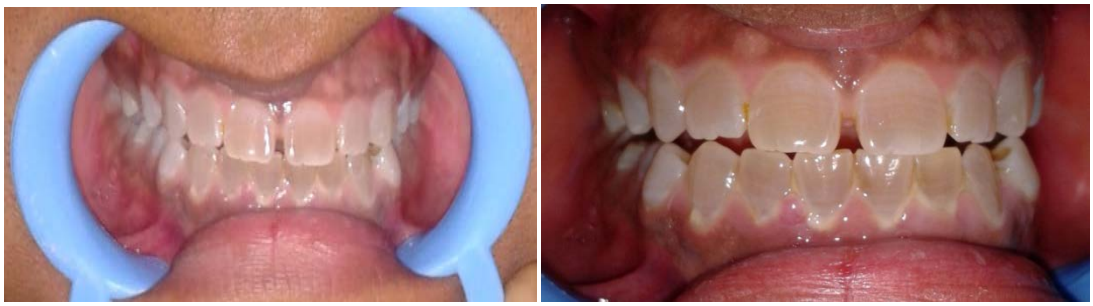

Figure (1a,1b): Generalized opalescent tooth discoloration.

Author α, σ, ρ ¥: Postgraduates Student Oral Medicine and Radiology Vyas Dental College & Hospital, Jodhpur Addresse-Priya Singhal, Shivalik Comlex, Flat A, Goal Buildng, Sardarpura, Jodhpur (Rajasthan). e-mail : prsinghal95@gmail.com,
Author ω: Postgraduates Student Oral & Maxillofacial Surgery Vyas Dental College & Hospital, Jodhpur.
Author §: Senior lecturer Oral Medicine & Radiology.

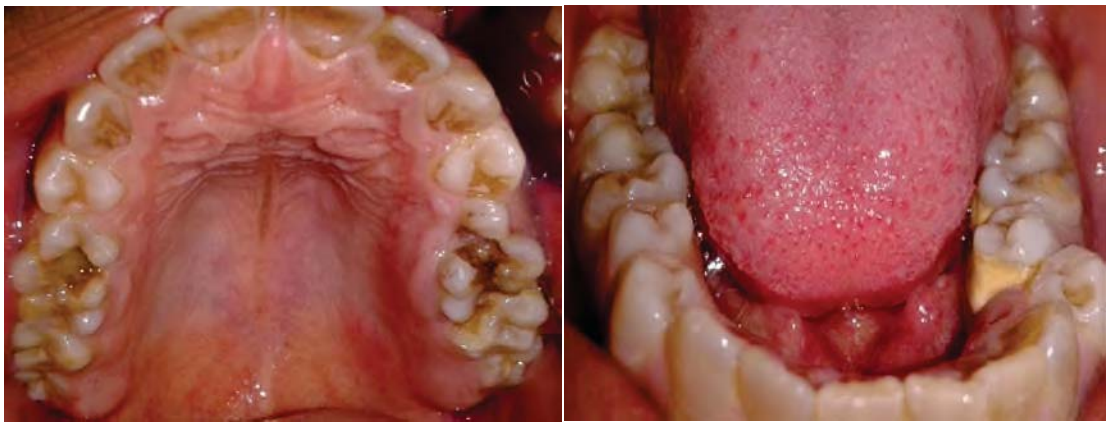

Figure (2a, 2b) : Intraoral examination revealed fractured cusps in multiple teeth

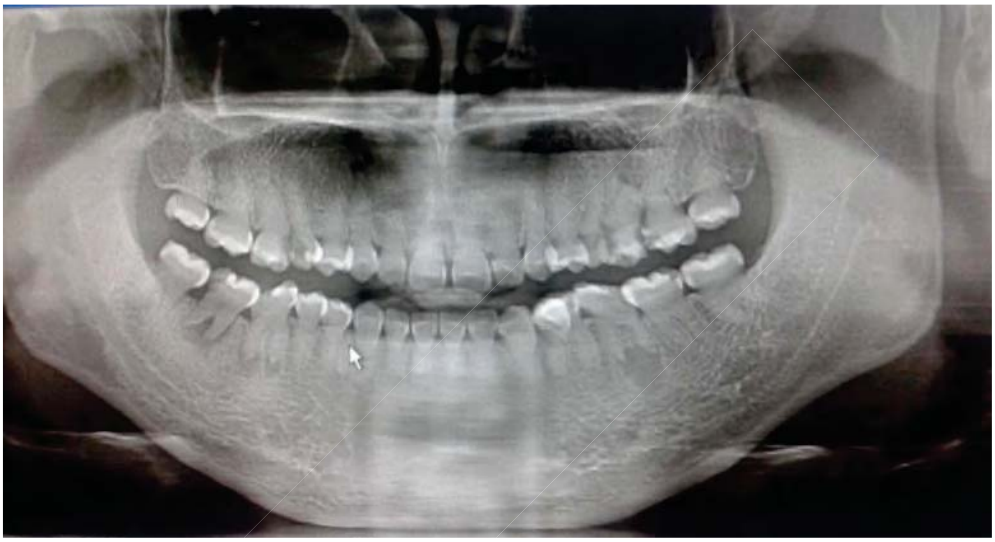

Figure 3a : OPG showing missing pulp chambers with complete Root obliteration of canals. Multiple periapicalradiolucencies are also seen.

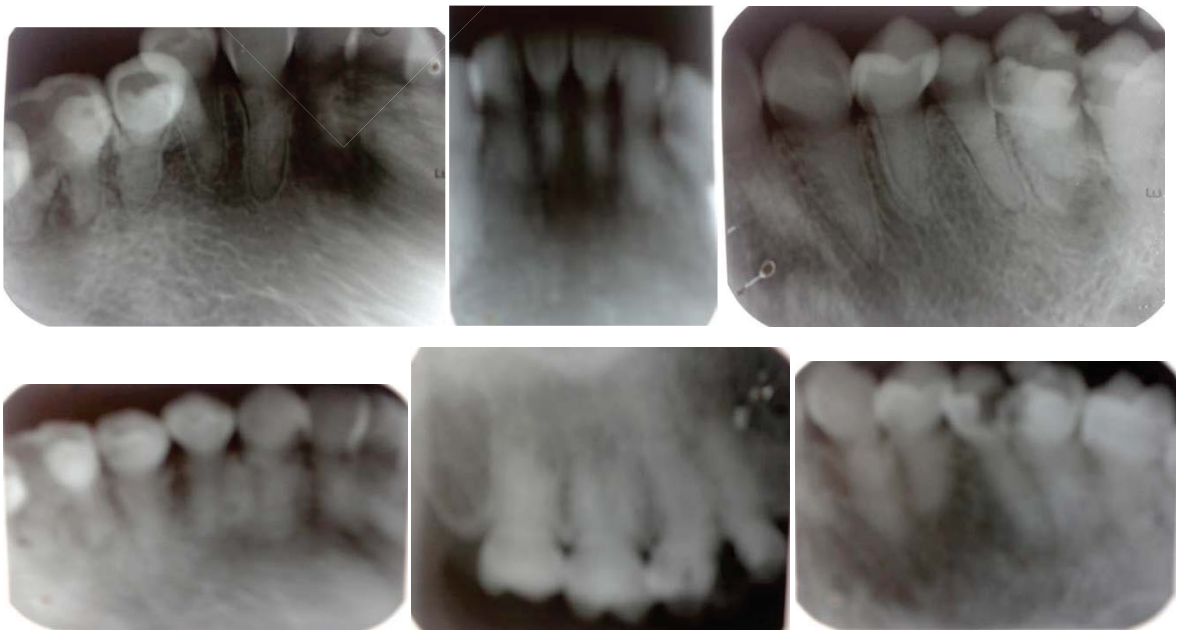

Figure 3b : Full mouth IOPAS showing missing pulp chambers

III. Discussion

Dentinogenesis imperfecta (DI) is one of the most common genetic disorders affecting the structure of dentin. It was recognized first time by Barret in1882 .In 1887 the condition was first described in a case involving a completely normal boy with dark staining on the teeth.[2] The term 'Dentinogenesis imperfecta' was coined by Robert and Schour in 1939.[3]

Witkop named the types Dentingenesis imperfecta, Hereditary opalascent dentin, and Brandywine isolate[4]. Sheild's et al classified Dentinogenesis imperfecta based on phenotypic variability into- Typel, Type II and Type III. Type I- occurs with Osteogenesis imperfecta. TypeII-DI not associated with Osteogenesisimperfecta; also known as hereditary opalescent dentin. Type III-DI of the "Brandywine type"[5]. Brandywine type (Dentinogenesis imperfecta 2) was found in the Brandywine triracial isolate in Southern Maryland. Multiple pulp exposures and "shell teeth" (due to large pulp chambers and thin dentinal walls) are two characteristics used to distinguish DI type III fromDI typeII. Genetic research has confirmed that Osteogenesis imperfecta with opalescent teeth clearly is a separate disease from Dentinogenesis imperfecta. Osteopontin,a bone glycoprotein is also expressed in dentin. However, there is no association between a type of polymorphism at the osteopontin locus and dentinogenesis imperfecta. Hence the following revised classification is proposed.

Dentinogenesis imperfecta1: Dentinogenesis imperfecta without osteogenesis imperfecta: The corresponds to dentinogenesis imperfecta type II of Shields classification.

Dentinogenesis imperfecta 2: Brandywine type dentinogenesis imperfecta: This corresponds to dentinogenesis imperfcta type III of Shields classification.

There is no substitute in the present classification for the category designated as DI type I of the Shields classification.

Clinically both the dentitions exhibit an unusual translucent, opalascent appearance with colour ranging from yellow-brown to grey. The entire crown appears discoloured because of the abnormal underlying dentin. Excessive constriction is present at the cementoenamel junction, giving the crowns a tulip or bell shape. Enamel is structurally and chemically normal but fractures easily which leads to rapid wear. The enamel fracturing is believed to be due to the poor support provided by the abnormal dentin and possibly in part to the absence of the scalloping normally seen between dentin and enamel that is believed to help mechanically lock the two hard tissues together[6]. The micohardness of the dentin closely approximates that of cementum, which also results in rapid attrition[8].Rapid wearing and absence of interdental contacts make the teeth less susceptible to caries. Dental tissues in DI will have low hardness, elasticity and stiffness leading to a phenomena of micromovement resulting in failure of restorations.[7]

Radiographically opacification of dental pulps occur in both the types I and II because of continued deposition of abnormal dentin.

Microscopically, dentin in dentinogenesis imperfecta contains fewer, but larger and irregular dentinal tubules. Pulp is nearly completely replaced over time by the irregular dentin. Enamel appears normal, but the DEJ is smooth instead of scalloped[6].

Dentin has two proteins in its composition: DSPP (dentinphospho protein) and dentin sialoprotein (DPS). DSPP is expressed in a number of tissues including bone, kidney, salivary gland and lung but its expression in dentine is hundreds of times higher than in other tissues Disturbances in the secretion of these proteins, and thus in the proper shape and placement of dental matrix crystals of apatite, manifested clinically as dentinogenesis imperfecta [9] .The genes responsible for producing both DPP and DSP are located at 4q12-21. Type I and Type III of DGI appear to result from mutations in the gene encoding DSPP suggesting that these conditions are allelic.[10]

Syndromes associated with dentinogenesis imperfecta are Osteogenesis imperfecta, Ehlers Danlos syndrome, Goldblatt syndrome, Schimkeimmunoosseousdysplasia, Brachio-skeleto-genital syndrome, Osteodys plastic primordial short stature with severe microdontia, opalescent teeth, and rootless molars[11]

Dentinogenesis imperfecta should be differentiated both clinically and radiographically from Amelogenesis imperfect (AI), Regional odonto dysplasia, Dentin dysplasia (DD), Tetracycline staining, Irradiation to jaws or chemotherapy during root development, Congenital erythropoietic porphyria and Dental Fluorosis.

Amelogenesis imperfecta like DI, is also a hereditary disorder. In AI teeth are usually sensitive and on radiographs enamel is less radio-dense and thinner than dentin. Pulp chamber and Root canals are usually not sclerosed.

Regional odontodysplasia is a localised anomaly restricted to a single tooth or a group of contiguous teeth while in dentinogenesis imperfecta all the teeth are involved. In Regional odontodysplasia the involved teeth either exhibit delayed eruption or do not erupt at all. Pulp chamber is very large giving a pale hazy image to the affected teeth, which is termed as ghost teeth.

Dentin dysplasia –Both DI and DD can produce crowns with altered colour and occluded pulp chambers. The finding of a 'thistle tube's haped pulp chamber in single rooted tooth strengthens the possibility of dentin dysplasia. The crowns in dentin dysplasia are usually of normal shape, size and

proportion while in dentinogenesis imperfecta teeth have bulbous shaped crowns with a constriction in the cervical region. If the roots are short and narrow, the condition is likely to be dentinogenesis imperfecta. On the other hand, normal appearing roots are present in dentin dysplasia type II or practically no roots at all in dentin dysplasia typeI12

`Congenital erythropoietic porphyria-It is a rare condition resulting from an inborn error of porphyrin metabolism. Abnormally high levels of porphyrin pigments are incorporated into teeth during their formation The entire primary and secondary dentitions are pink or reddish brown. Under ultraviolet light, the teeth fluoresce red13.The teeth discolouration is usually found at the necks of teeth and the enamel hypoplasias are usually located in coronal third of the teeth and no pulp sclerosis is seen while in DI pulp sclerosis is present.

Tetracycline staining-The erupting affected teeth have a bright yellow band-like appearance that fluoresces under ultraviolet light. On exposure to sunlight, the colour gradually changes to grey or red-brown. Radiographically there is no pulp sclerosis in tetracycline staining while in Dentinogenesis imperfecta pulp sclerosis is present.

Dental Flourosis-Ingestion of drinking water containing fluoride at levels greater then 1 ppm during the time crowns are being formed may result in enamel hypoplasia or hypocalcification or fluorosis. Mild to moderate fluorosis ranges clinically from white enamel spots to mottled brown and white discolorations. Severe fluorosis appears as pitted, irregular and discoloured enamel. No pitting is seen in dentinogenesis imperfecta and also crown gives opalescent appearance.Pulp obliteration is seen in dentinogenesis imperfecta which is absent in dental fluorosis.

IV. Conclusion

Dentinogenesis imperfecta causes esthetic as well as functional problems to the patients.Where diagnosis occurs early in the life of the patient,good aesthetics and function can be obtained thereby minimising nutritional deficits and psychosocial distress.To give a diagnosis of dentinogenesis imperfecta syndromes related to it and other causes of teeth discolouration should be taken into consideration.

References Références Referencias

1. Martin J Barron, Sinead T McDonnell, Jain Mackle, Michael J Dixon. Hereditary dentine disorders: dentinogenesis imperfect and dentin dysplasia: Review. Orphanet Journal Of Rare Diseases 2008; 3:31.
2. Nayeir AK. Laua IB, Soni NN, Treatment of dentinogenesis imperfecta in a child: report of a case.Dem Child 1981; 48:453-455.
3. Kamboj, Anil Chandra. Dentinogenesis imperfect type II: an affected family saga. Journal of Oral science 2007; 49: 241-244.
4. Witkop CJ. Hereditary defects in enamel and dentin. Acta Genet 1957; 7:236-239.
5. Shields ED, Bixler D, el-Kafrawy AM. A proposed classification for heritable human dentine defects with a description of a new entity. Arch Oral Biol 1973;18: 543-553.
6. Regezi, Sciubba, Jordan. Oral Pathology Clinical Pathologic Correlations;pg-371.
7. De Coster, P.J., Cornelissen M., de Paepe A, Martens L. C., Vral. A., Abnormal dentin structure in two novel gene mutations [COL1A1. Arg134Cys] and [ADAMTS2, Trp795-to-ter] causing rare type I collagen disorders, Arch. Oral Biol 2007; 52:101–109.
8. Aneta Wieezorek,Jolanta Loster. Dentinogenesis imperfect TypeII: Ultrastructure of teeth in sagittal sections: Original study. Polia Histochemica Et Cytobiologica 2013; 51: 244 -247.
9. Aneta Wieezorek, Jolanta Loster, Wojciech Ryniewicz, Anna M. Dentinogenesis imperfecta – hardness and Young's modulus of teeth. Acta of Bioengineering and Biomechanics Original paper 2013;15,No.3.
10. Shafer, Hine, Levy. Shafer's Textbook of Oral pathology, New Delhi: Elsevier, 2006;pg- 55.
11. Kantaputra PN: Dentinogenesis imperfect-associated syndromes. Am J Med Genet 2001;104:75-78.
12. White and Pharaoh. Oral Radiology: Principles and Interpretation, New Delhi:Elsevier,2011;pg-310
13. BK Venkataraman. Diagnostic Oral Medicine, New Delhi:WoltersKluwer,2013;pg-114-116

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Duplex Ultrasound, Clinical Score, and D-Dimer to Rule in and Out Deep Venous Thrombosis (DVT) and Postthrombotic Syndrome (Pts): Bridging the Gap between DVT and Ptsin the Primary Care and Hospital Setting

By Jan Jacques. Michiels, Janneke M. Michiels,Hannie Maasland, Mildred Lao, Wim Moosdorff, H.A. Martino Neumann & Gualtiero Palareti

Leiden University Medical Center , Netherlands

Abstract- First episodes of DVT in adults and elderly are elicited in two-thirds of cases by risk factors, including varicose veins, cancer, pregnancy/postpartum, oral contraceptives below the age of 50 years, immobility or surgery. Pain and tenderness in the calf and popliteal fossa may occur resulting from conditions labeled as alternative diagnosis (AD) including Baker's cyst, hematoma, or muscle tears or pulls. The requirement for a safe diagnostic strategy of deep vein thrombosis (DVT) should be based on an objective post-test incidence of venous thromboembolism (VTE) of less than 0.1% with a negative predictive value for exclusion of DVT of 99.90% during 3 months follow-up.

Keywords: deep venous thrombosis, ultra sonography, post-thrombotic syndrome, elisa d-dimer, medical elasticstockings, anticoagulation.

GJMR-D Classification : NLMC Code: WB 289

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Duplex Ultrasound, Clinical Score, and D-Dimer to Rule in and Out Deep Venous Thrombosis (DVT) and Postthrombotic Syndrome (Pts): Bridging the Gap between DVT and Ptsin the Primary Care and Hospital Setting

Jan Jacques. Michiels [α], Janneke M. Michiels [σ], Hannie Maasland [ρ], Mildred Lao [ω], Wim Moosdorff [¥], H.A. Martino Neumann [§] & Gualtiero Palareti [χ]

Abstract- First episodes of DVT in adults and elderly are elicited in two-thirds of cases by risk factors, including varicose veins, cancer, pregnancy/postpartum, oral contraceptives below the age of 50 years, immobility or surgery. Pain and tenderness in the calf and popliteal fossa may occur resulting from conditions labeled as alternative diagnosis (AD) including Baker's cyst, hematoma, or muscle tears or pulls. The requirement for a safe diagnostic strategy of deep vein thrombosis (DVT) should be based on an objective post-test incidence of venous thromboembolism (VTE) of less than 0.1% with a negative predictive value for exclusion of DVT of 99.90% during 3 months follow-up. Complete duplex ultrasonography (CDUS) does pick up AD not only Bakers cyste but also muscle hematomas, old DVT, and superficial vein thrombosis (SVT). AD with a negative CDUS include leg edema, varices erysipelas are picked up by physical examination. The sequential use of DUS, a sensitive D-dimer test and objective clinical score assessment is a safe cost-effective non-invasive strategy to rule in and out DVT and AD in patients with suspected DVT.

A first DVT event in adults and elderly has a gradually increasing 20 to 30% DVT recurrence rate after 5 years follow-up. Research studies show that complete recanalization on DUS within 1 to 3 months and no reflux is associated with with low recurrence of DVT (1.2% patient/years) and withno PTS obviating the need of MECS and anticoagulation after 3 to 6 months post-DVT respectively. Delayed recanalization at 3 months post-DVT and the presence of reflux due to valve destruction on DUS at 3 months post-DVT is associated with a high risk of DVT recurrence as the cause of symptomatic PTS indicating the need to wear MECS and extend anticoagulation. Delayed recanalization, previous SVT, presence of varicosis, reflux, abnormalties on CUS, and increase of D-dimer above normal after discontinuations of anticoagulant treatment are predictors of DVT recurrence and candidates for extended use of low dose NOACs. We designed a prospective safety-outcome study bridging the gap between DVT and PTS with the aim to reduce the overall DVT recurrence rate to less than 3% patient/years during 5years follow-up.

Keywords : deep venous thrombosis, ultra sonography, post-thrombotic syndrome, elisa d-dimer, medical elasticstockings, anticoagulation.

Author α σ: Hemostasis Thrombosis Research Vascular Medicine Science Center. e -mail: goodheartcenter@upcmail.nl

Author σ ρ : Goodheart Institute Rotterdam and Medical Diagnostic Center Rijnmond.

Author σ ρ: Rotterdam Primary Care Medicine, Leiden University Medical Center, Leiden.

Author σ ω: The Netherlands. Department of Dermatology, Section Phlebology.

Author σ ¥ : Erasmus University Medical Center, Rotterdam.

Author ω § : The Netherlands, Department of Angiology & Blood Coagulation University Hospital.

Author ¥ χ : Policlinico S. Orsola Malpighi, Bologna, Italy.

I. Introduction Deep Vein Thrombosis: DVT

First-time episodes of deep vein thrombosis (DVT) are in two-thirds of cases caused by risk factors, including varicosis, cancer, immobility, or surgery, oral contraceptives and thrombophilia (table 1)[1,2]. Alterations in blood coagulability with changes in blood flow and endothelial damage, are the precursors of intravenous thrombosis. A number of other hereditary and acquired conditions that predispose to thrombosis (thrombophilia) have been recognized. These include protein C and S deficiency, antithrombin III deficiency and activated protein C resistance (which is usually associated with a factor V genetic abnormality[3]. Screening for these thrombophilia and for anticardiolipin antibody, should only be performed in patients having familial, sporadic or recurrent thrombosis.

Table 1:

Characteristics of 1668 patients with a first deep vein thrombosis (DVT) subdivided in calf vein 203 (17%), polpiteal vein 498 (41%) and femoral politieel 502 (42%) Search for new risk factors First DVT anno 2012 Tick et al JTH 2008

Main Characteristics: Risk factors for 1st DVT	Number	%
• Varicose veins at diagnosis	470	28
• Malignancy	137	8
• Surgery <3months	357	21
• Minor injury, Plaster cast <3months	405/105	24+6
• Oral anticonceptive pill (OAC) <50 yrs	*417 (74%)*	*25*
• Hormonal replacement >50 yrs	39	13
• Idiopathic DVT	389	24
• FV Leiden mutation	315	19
• Prothrombin 20210A mutation	92	6
• Venous Thrombophilia FV Leiden & FII 20210		25

Conclusion from Tick et al JTH 2008;6:2075-2081
Change from Surgery, Malignancy & Immobilozation (primary VTE prevention) to Minor Injury, Plaster Cast and OAC (no primary VTE prevention) as eliciting factors for 1st DVT. Michiels 2012 Lecture World IUA Congress Prague

II. Clinical Features and Diagnosis of DVT

The onset of a thrombosis is often 'silent' and may remain so. It commonly occurs at or about day 7 to 10 after a surgical operation, parturition or the onset of an acute infection. Between one-third and two-thirds of patients complain of some swelling and pain in the leg, usually in the calf[1-3]. An iliac thrombosis should be suspected if the whole leg is swollen and dusky. Direct pressure on the calf muscles or over the course of the deep veins usually elicits direct tenderness. There may be a cyanotic hue to the leg and superficial venous dilatation. The temperature of the leg may be raised, and oedema of one ankle is an important physical sign. However, chest pain or cardiac arrest from pulmonary embolism may be the first indications of a DVT. Pulmonary hypertension may follow repeated small emboli, and is associated with the development of progressive dyspnoea.

Pain and tenderness in the calf and popliteal fossa may occur resulting from alternative diagnoses (AD) like conditions such as a ruptured Baker's cyst, a torn plantaris tendon, a hematoma, or muscle tears or pulls. Cutaneous infection (e.g. cellulitis), lymphoedema, venous reflux, peripheral arterial disease, and rheumatological causes should also be differentiated from DVT.

As compared with phlebography (the reference gold standard to exclude and diagnose proximal DVT in prospective management studies), the sensitivity of compression ultrasonongraphy (CUS) is 97% for proximal and 73% for distal vein thrombosis[4,5]. CUS has many advantages over phlebography. It is noninvasive, simple, easy to repeat, relatively inexpensive, and free of complications. However, there are two main disadvantages of CUS. First, calf vein thrombosis will be overlooked by CUS because it is safe to limit CUS estimation to the subpopliteal, popliteal, and femoral veins for the diagnosis of symptomatic proximal DVT. Second, isolated thrombi in the iliac and superficial femoral veins within the adductor canal are rare but difficult to detect and therefore easily overlooked in symptomatic patients with suspected DVT (10).

Estimates of clinical score as low, moderate, and high for the probability of proximal DVT, based on medical history and physical examination is the first step when DVT is suspected (Table 2)[4,5]. A score of 0 (asymptomatic) means a low probability, a score of 1 or 2 a moderate probability, and a score of 3 or more a high probability for DVT.

Clinical feature	Score
Active cancer treatment ongoing or within previous 6 months or palliative	1
Paralysis, paresis, or recent plaster immobilization of the lower leg(s)	1
Recent immobilization for more than 3 days or major surgery within last 4 weeks	1
Localized tenderness/pain along the distribution of the deep venous system	1
Entire leg swollen	1
Calf swelling by more than 2 cm when compared with the asymptomatic leg (measured 10 cm below tibial tuberosity)	1
Pitting oedema greater in the symptomatic leg	1
Collateral superficial veins (nonvaricose)	1
Total Rotterdam DVT score	**8**

Tabel 2 : Clinical score list for predicting pretest probability for proximal DVT The Rotterdam modification of the Wells' score[4,5].

D-dimer is a degradation product of a cross-linked fibrin clot. It has gained a prominent role for ruling out DVT. The prevalence of DVT after a negative first CUS is uniformly low, 2 to 3%, during a 3 months follow-up with a NPV of 97 to 98% in large prospective management studies (figure 1)4. The combination of a negative first CUS and a negative SimpliRed safely excludes DVT to zero or less than 1% (NPV > 99.3 to 100%) irrespective of clinical score (figure 1) (11). This simple, safe and most effective strategy obviates the need of repeated CUS on the basis of which anticoagulant therapy can safely be withheld and reduces the number of repeated CUS testing from about 75% to 30% (figure 1)[6].

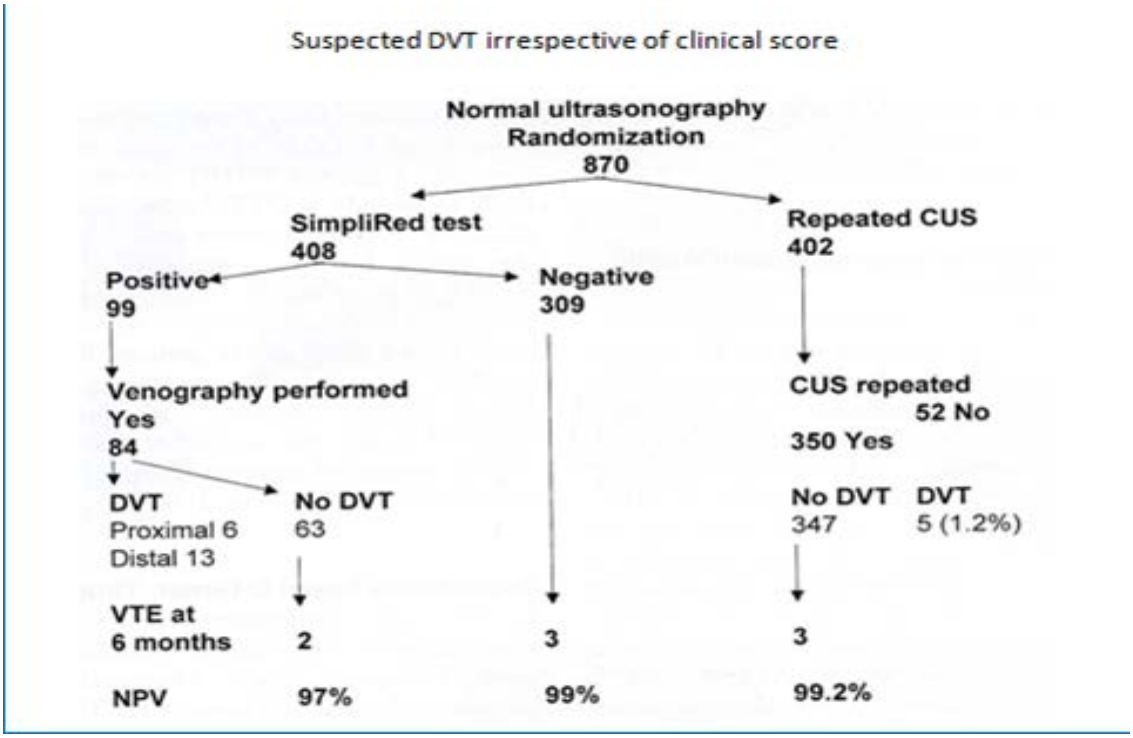

Figure 1 : Safe exclusion and diagnosis of deep vein thrombosis by the sequential use of CUS and SimpliRed test in the primary care setting or outpatient ward[6]

The negativepredictivevalue of a normalqualitative D-dimerSimpliRedtest is not high enoughfor the exclusion of DVT[4,5]. After a negativefirst CUS and a negativeSimpliRed (Simplify) test DVT isexcludedwith anegativepredictivevalue of 99.0% with the needtorepeat a second CUS when the D-dimer is positive (Figure 1)[6].

The feasibility of a strategytoexclude DVT by a negativerapid ELISA VIDAS D-dimer test (<500 ng/ml) alone without the need of CUS, on the basis of whichanticoagulant treatment is withhold, has been tested in the large prospective management Geneva study (table3)[6] In thisGeneva study of 474 outpatientswithsuspected DVT, the sensitivity and negativepredictivevalue(NPV) of a rapid ELISA D-dimer test forthrombosisexclusionwere 98.2 and 98.4% respectively (table 3). A negative CUS followedby a negative ELISA VIDAS D-Dimertest result (<500 ng/mL) exclude DVT and pulmonaryembolismwith a sensitivity, and NPV of 100% irrespective of clinical score (table 3 left arm, Geneva Study)[7].

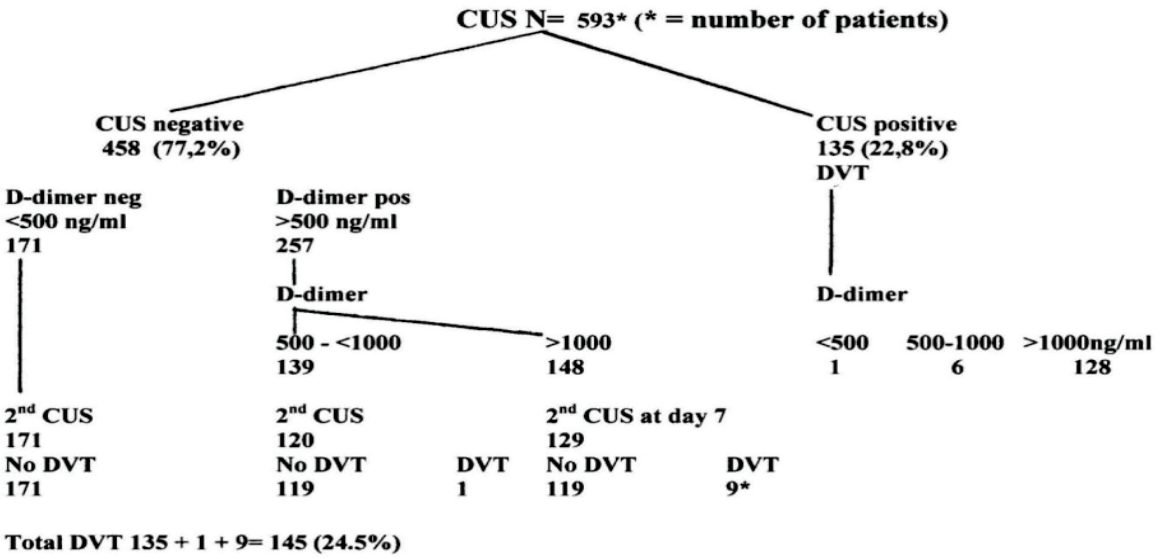

Table 3 : Results of CUS and ELISA D-dimertesting in 593 patientswithsuspected DVT in the prospective Geneva management study[7].

We tested the sequential use of the sensitive quantitative ELISA VIDAS D-dimer test, clinical score, and compression ultrasound (CUS) as a safe and cost-effective diagnostic work-up of DVT in a large prospective management study according to the diagnostic algorithm in figure 2A. According to figure 2A DVT is excluded after a negative CUS and normal VIDAS D-Dimer (<500 n g/m L) with a sensitivity and NPV of 100%. If the first CUS is negative and the VIDAS D-dimer increased (>500 n g/m L) a second CUS was performed within one week.

Out of 865 outpatients with suspected DVT, a 1st CUS was negative in 675 , and positive in 190 (22%). In 249 outpatients with a normal ELISA VIDAS (<500 n g/m L) the CUS was true negative in 248 and false negative in 1 (sensitivity 99.5%, figure 2B). The negative predictive value of a first CUS was 98.6% (662 out of 675) on the basis of which a second CUS within one week should beper for med. A 2nd CUS was indicated in 427 and performed in 382 because of increased ELISA VIDAS D-dimer (>500 n g/m L) and proved to be positive in 13 (1.9% of 381).

The overall prevalence of DVT in o rprospective management study was 190 plus 13 = 203 (23.4%, figure 2 B). The prevalence of DVT on CUS at increasing ELISA VIDAS cut off levels of 0 to 500 (N=249), 500 to 1000 (N=196) and above 1000 n g/m (N=374) was 0.5%, 5.1% and 19.5%.

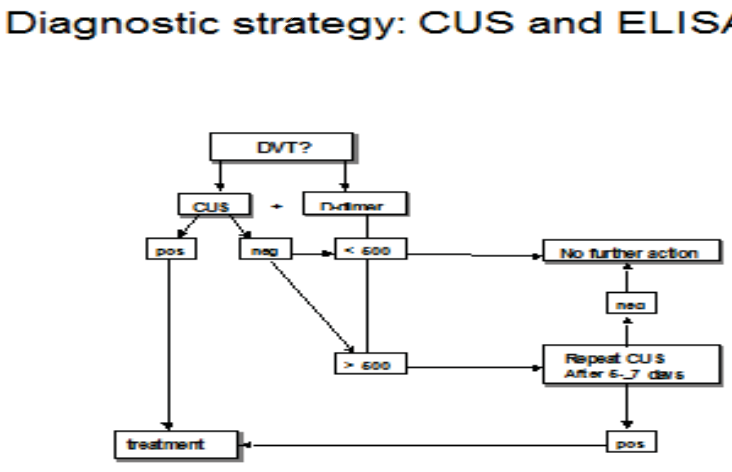

Figure 2 A : The Study design in patients with suspected deep vein thrombosis (DVT) in the Primary Care Setting of the Medical Diagnostic Center Rotterdam 1998-2005

A normal ELISA VIDAS D-dimer test (<500 ng/mL) aloneex cluded DVT at a sensitivity and NPV of 99.5% and a specificity of 37.5% (figure 2 B).

The sensitivit y for DVT exclusion of an ELISA VIDAS test result between 500 and 1000 n g/m L after a first negative CUS was 99.6 as test edbyrepeated CUS. The combination of a first negative CUS and an ELISA VIDAS test between 500 and 1000 n g/m L will exlude DVT with a NPV of 99.7% (figure 2B).

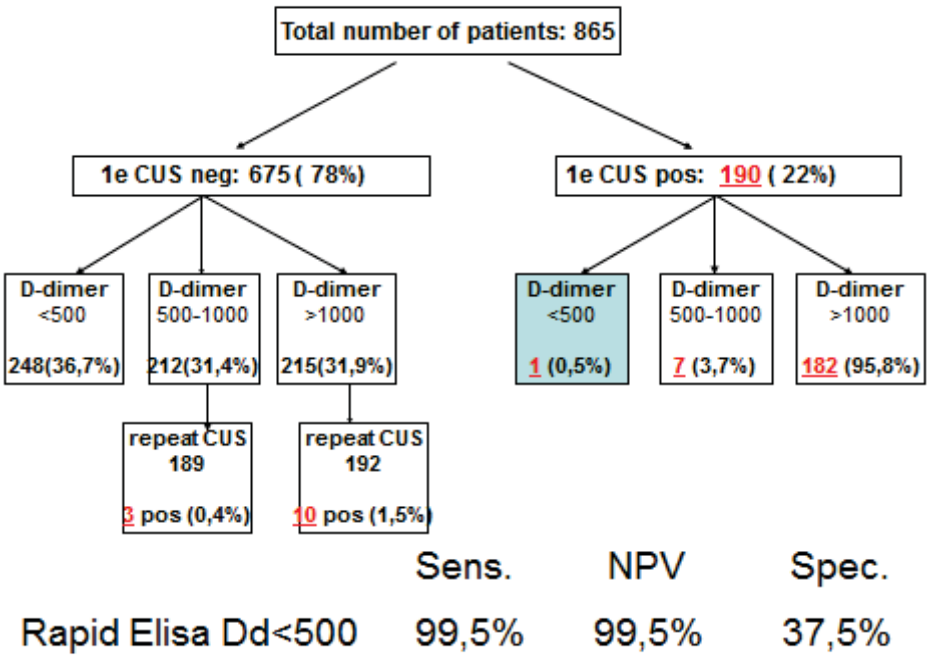

Figure 2 B : Result of the Rotterdam prospective management study according to the diagnostic design to rule in and out DVT in outpatients with suspected DVT in figure2A

From this prospective management study we concluded that the combined use of a sensitive quantitative ELISA D-dimer test, clinical score, and compression ultrasound (CUS) is a safe and cost-effective diagnostic work-up to exclude and diagnose according to the algorithm in figure 3. First, it is safe to exclude DVT and pulmonary embolism by a negative CUS and normal ELISA D-Dimer test (<500 n g/ml, left arm) with a sensitivity and NPV of 100%. Second, it is safe to exclude DVT by a first negative CUS and an ELISA VIDAS D-Dimer test result of <1000 n g/m L in asymptomatic cases with a low clinical score of zero (middle arm) with a sensitivity and NPV of 99,6%. Third, attentive clinicians should be aware that the combination of a negative first CUS and an ELISA D-Dimer test above 500 n g/m L in patients with persistent complaints, signs and symptoms suspected for DVT are candidates to perform a second CUS (right arm, figure 3). This new strategy in figure 3 is predicted to l reduce repeaedt CUS testing by two-third, which has been tested in a large prospective management study of more than 2000 patients with suspected DVT between 2006 and 2013 (manuscript in preparation).

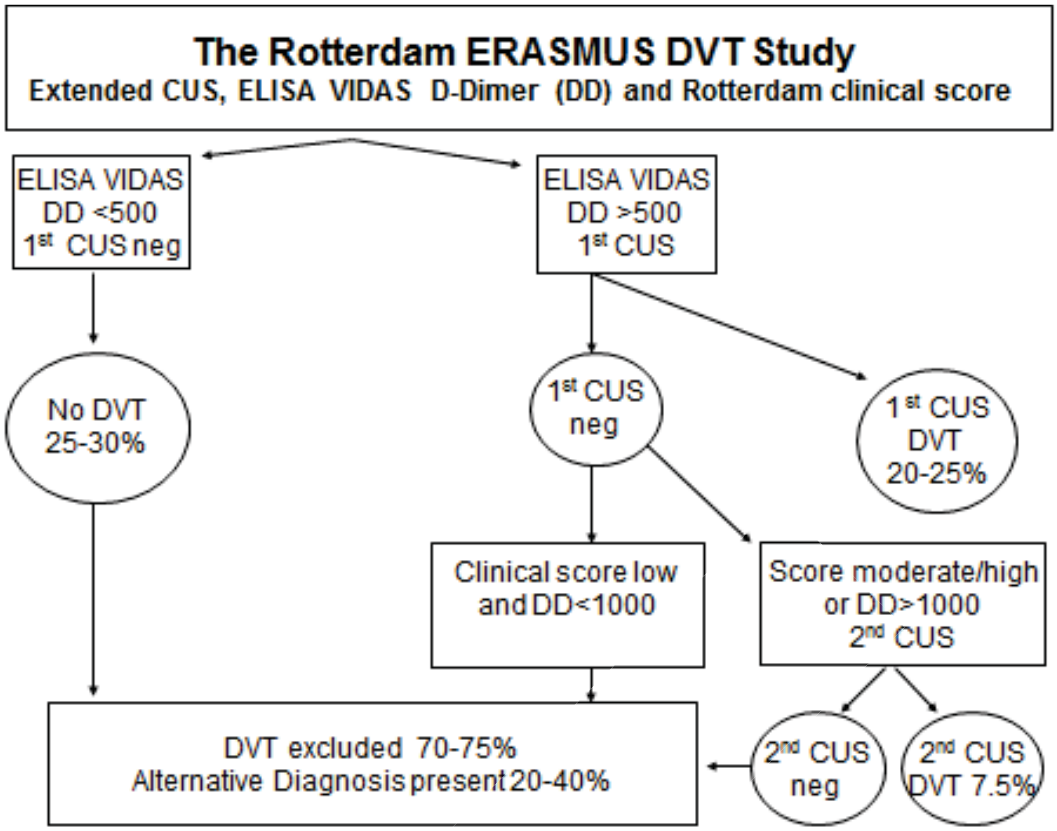

Figure 3 : The Rotterdam approach of diagnosis and exclusion of DVT by CUS, ELISA VIDAS D-Dimer testing and clinical score assessment between 2006 and 2014[4,5].

The sensitivity of a normal (<500 ng/ml) turbidimetric D-dimer assay Tinaquant (Roche Diagnostics, Marburg Germany) for the exclusion of DVT in a large management studywas 97.7% at a specificity of 42% and a NPV 97% (figure 4)8. A normal Tinaquant test result (<500 ng/ml) as a stand alone test appearstobe not sensitiveenough for the exclusion of DVT. The combination of a negative first CUS, a normal Tinaquant test result (<500 n g/ml) and a low clinical score had a negative predictive value of 99.4% without the need of CUS testing and thusreduced the number of CUS testingby 29% (Figure 4)[8]. Those patientsa first negative CUS, a Tinaquant around and above 500 n g/m L and persistent symptom ssuspected for DVT (moderate/high clinical score) are candidate for CUS testing within one week toexclude or diagnose DVT or analternative diagnosis in the primary care setting.

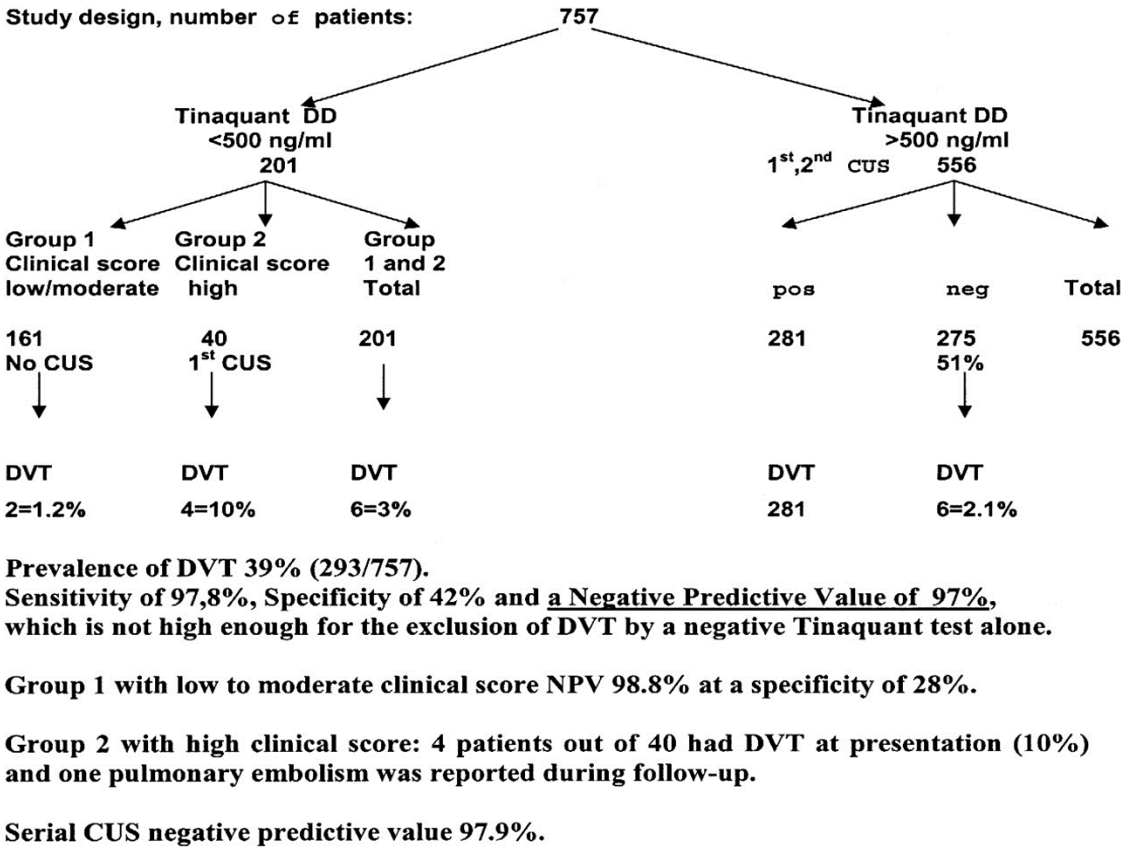

Figure 4 : Result of a prospective management study according to the diagnostic strategy to rule in and out DVT in outpatients with suspected DVT by Tinaquant D-Dimer testing and CUS[8].

Oudega et al compared the cut-off values for the ELISA VIDAS and the turbidometric Tinaquant D-Dimer assays in a prospective study among 110 primary care physicians in a large population of 358 and 330 patients with suscpected DVT respectively[9]. To safely exclude DVT, a high sensitivity and NPV are needed to limit missed DVT cases. At the 500 n g/m L threshold, the ELISA VIDAS assay did meet this criterion (table 4A) in contrast to the Tinaquant. For the Tinaquant D-Dimer assay lower threshold are preferred for excluding DVT in the primary care setting (table 4B).

Table 4 A : Diagnostic accuracy of the ELISA VIDAS D-Dimer assay at different cut-off values for the exclusion of deep vein thrombosis (DVT) in 358 patients with suspected DVT in the primary care setting

D-dimer cut-off value (ng/ml)	DVT absent*	DVT present*	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)
100	5	1	99	1	83
200	23	1	99 (96 - 100)	6 (4 - 9)	96 (79 - 100)
300	47	1	99 (96 - 100)	13 (10 - 17)	99 (89 - 100)
400	69	1	99 (96 - 100)	19 (15 - 23)	99 (92 - 100)
500	103	1	99 (96 - 100)	29 (24 - 34)	99 (95 - 100)
600	123	2	97	34	98
700	141	2	97	39	99
800	156	2	97 (93 - 100)	44 (38 - 49)	99 (96 - 100)
900	173	3	96	48	98
1000	199	8	88	56	96
1100	220	8	88	62	97
1200	241	9	87	67	96
1300	254	10	85	71	96
1400	264	10	85	74	96
1500	277	11	84	77	96
all patients	**358**	**67**			

Table 4 B : Diagnostic accuracy of the turbidometric Tinaquant D-Dimer assay at different cut-off values for the exclusion of deep vein thrombosis (DVT) in 358 patients with suspected DVT in the primary care setting

D-dimer cut-off value (ng/ml)	DVT absent*	DVT present*	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)
100	24	1	99	7	96
200	68	2	98 (95 - 100)	21 (16 - 25)	97 (95 - 100)
300	109	5	95 (90 - 99)	33 (28 - 38)	96 (90 - 99)
400	136	6	94 (89 - 99)	41 (36 - 47)	96 (94 - 98)
500	167	9	91 (85 - 97)	51 (45 - 56)	95 (91 - 98)
600	189	11	89	57	95
700	202	12	88	61	94
800	216	14	84 (76 - 91)	65 (60 - 70)	93 (89 - 96)
900	220	19	80	67	92
1000	227	22	77	69	91
1100	230	23	76	70	91
1200	235	24	75	71	91
1300	244	27	72	74	90
1400	251	28	71	76	90
1500	258	29	70	78	90
all patients	**330**	**97**			

At time of a 1st negativec CUS in our prospective management study (figures 2A and 2B), we found thatthe CUS was positive for analternative diagnosis (AD) including Bakers cyste, musclehematoma or old DVT in 6.7%, for superficial throm bophlebitis without DVT in 7.8%, and leg edema in 17%, indicating the need to start with CUS followed by D-Dimer testing and clinical score assessment to increase the efficacy of the diagnostic calgoritm in figure 3 [4,5]. This strategy is currently under investigation in a large prospective management study of more than 2000 patients with suspected DVT between 2006 and 2014 (manuscript in preparation).

We emphasize that DVT should be diagnosed and excluded based on a non-in vasie strategy with an objective post-test incidence of venous throm boembolism (VTE) of lessthan 0.1% with a negative predictive value of 99.5% to 99.9% during 3 months follow-up[10].

Modification of the Wells score bye limination of the "minus 2 points" for AD is mandatory and will improve the diagnostic caccuracy of DVT suspicion significantly in the primary care setting and outpatient ward [4,5,10]. The sequentialuse of complete DUS first, followed by ELISA D-Dimer testing and modified clinical Wells' score assessment is safe and effective for the exclusion and diagnosis of DVT and AD.

III. Introduction Posthrombotic Syndrome: PTS

The postthrombotic syndrome (PTS) is a chronic condition that affects the deep venous system, and may also extend to the superficial venous system of the legs in patients with a documented history of deep vein thrombosis (DVT) [11-13]. Clinical symptoms of PTS may vary considerably and range from scarcely visible skin changes to changes in pigmentation, pain, discomfort, venous ectasia, oedema, and ulceration. Patients experience pain, heaviness, swelling, cramps, itching or tingling in the affected limb. Symptoms may be present in various combinations and may be persistent or intermittent. Typically, symptoms are aggravated by standing or walking and improve with resting, leg elevation and lying down. Next to varicose veins, a specific sign of early forms of venous incompetence is the so-called corona phlebectatica para plantaris (ankle flare). There are telangiectases surrounding the malleoli of the ankle. Other signs are oedema, hyperpigmentation, and eczema. Signs of more advanced disease are dermato- and liposclerosis, a localized induration of the skin and sometimes of the underlying tissues, with fibrosis and inflammation. Through microthrombi, an area of whitened skin with reddish spots may occur, called atrophie blanche or white atrophy. The most severe sign is the venous leg ulcer, a chronic wound that fails to heal spontaneously or within the time range of normal healing of the skin.

DVT has a recurrence rate of about 20% to 30% after 5 years, but the rate varies depending on the presence of risk factors for thrombosis recurrence and particularly increases after discontinuation of prolonged anticoagulation[11].The only clear identified risk factor for overt PTS is recurrent or ipsilateral DVT, which increases the risk of PTS several fold[11-13.] In a prospective study of 313 consecutive DVT patients, Prandoni et al have shown that Residual Vein Thrombosis (RVT) at any time post-DVT is a risk factor for recurrent VTE. In this study, DUS of the common femoral and popliteal veins was performed at 3, 6, 12 24 and 36 months post DVT. The cumulative incidence of RVT was 61%, 42%, 31%, and 26% at 6, 12, 24 and 36 months (1/2, 1, 2 and 3 years) post DVT respectively. Of 58 VTE recurrent episodes, 41 occurred at time of RVT. The hazard ratio for recurrent VTE was [2.4] with persistent RVT versus those with earlier complete vein renalization2. The post-DVT re-canalisation process in patients with acute proximal DVT is completed between 3 months to 2 years, depending on the severity of DVT and the presence or absence of transient or persistent risk factors for thrombosis recurrence and PTS. This observation is in favor to extend anticoagulation at 6 months and 1 year post-DVT when RVT on DUS is present in symptomatic PTS patients during follow-up for about 2 to 3 years or even life-long post-DVT.

IV. Classification of PTS

The CEAP (Clinical-Etiology-Anatomic-Pathophysiologic) (table 5) for PTS is the best known and widely used by phlebologists but not by clinicians or vascular medicine specialists[3]. In 1992, Prandoni developed an original standardized scale for the assessment of PTS in post-DVT legs. Each subjective sign and objective symptom was scored as 0, 1, 2 or 3 based on subjective judgement[13]. The 5 signs and 7 symptoms of the Prandoni score are taken over by PTS investigators as the Villalta in the literature exactly as first designed by prandoni for use in clinical practice (table 6)[11]. The original Prandoni (subjective Villalta) score of signs and symptoms has never been evaluated against the objective CEAP scoring system as we proposed in table 3. It is mandatory to validate both the Prandoni (Villalta) scoring of signs and symptoms of early PTS in the modern setting of PTS evaluation against objective scoring system in table 3 at 1, 3, 6 and 12 months up to 2 years post-DVT. This statement is of particular interest, since literature data are convincing in showing that PTS is present in about 50% one year post-DVT using the Villalta score as compared, and does not increase in frequency and severity when under continuous medical care.

Table 5 A : Clinical part of the CEAP classification for severity of chronic venous insufficiency either primary or secondary see E, A and P parts: EAP)[13]

Classification	Symptom
Clinical: C C0 asymptomatic	 No visible varicose veins

C1	Spider or reticular veins
C2	Varicose veins
C3	Oedema
C4a	Pigmentetion or eczema
C4b	Lipodermatosclerosis or atrophie blanche
C5	Skin changes with healed ulceration
C6	Skin changes with active ulceration
Symptomatic	Symptomatic, including aches, pain, tightness, skin irritation, heaviness, muscle cramps, and other complaints attributable to venous dysfunction
A	Asymptomatic

Table 5 B : The Etiologic, Anatomic-Pathophysiologic (EAP) part of the CEAP classification for severity of chronic venous insufficiency[3]

E = Etiology	Congenital, primary, secondary, post-DVT = PTS
A = Anatomic distribution	Deep, perforator, or superficial vein, alone or in combination. Not taken into account in CEAP in PTS
P = Pathophysiologic dysfunction	Reflux or obstruction, alone or in combination. P is not part of CEAP in PTS evaluation

Table 6 : Clinical scoring system according to Prandoni for the assessment of early PTS in the early period of 3 to 12 months post-DVT up to 2 years post-DVT[13]

5 Subjective symptoms: Identical to S of CEAP	*7 Objective sign :*	All belong to C of CEAP[15]
Heaviness of the foot/leg	Pretibial oedema	C3
Pain in calf/thigh	Induration of the skin	C4
Cramps in calf/thigh	Hyperpigmentation	C4
Pruritus	New venous ectasia	C2
Paraesthesia	Redness	
Pain during calf compression (post DVT)		
Ulceration of the skin	C5, C6	

Each sign or symptom is graded with a score as 1, 2, or 3.
(1 = mild, 2 = moderate, 3 = severe). Ulceration of the skin 4 points
The presence leg ulcer is a late complication of PTS consistent with severe CVI, CEAP 5, 6.

Definition of clinical post-t romb tic syndrome according to Prandoni (Villalta)
Absent: score <5
Mild-to-moderate: score between 5 and 10 and 10 to 15 respectively at 2 consecutive visits
Severe: score ≥15 at 2 consecutive occasions
Source: Pesavento R, Prandoni P. Phlebology Digest 2007;20:4-8
Conclusion: the Prandoni scoring system is an ordered use of CEAP clinical symptoms and signs (C&S) by internist without expert dermatological evaluation and objective testing with CUS. Moreover this scoring system should be used at 1, 3, 6, 9 months and 1 years pos-DVT for the assessment of disappearance or persistence of DVT symptoms and the appearance of mild, moderate and severe PTS symptoms

V. Normal Versus Abnormal DUS at 3 Months Post-DVT

The study of Prandoni et al[14] have clearly demonstrated that about half of the DVT patients do not develop PTS in the control group, and that compression therapy using medical elastic compression stockings (MECS) decreases this incidence with 50% (from circa 50 to 25% indicating an absolute reduction rate of 25%) during an observation and treatment period of 2 years following DVT. Family doctors, phlebologists and internists usually treat the acute phase of DVT with anticoagulants for at least 3 to 6 months[1-4]. Physicians with interest and expertise in phlebology are usually confronted much later and frequently too late with overt post-thrombotic complications of DVT[11-14].

In the study of Siragusa et al [15], residual vein thrombosis was detected in 180 (69, 8%) of 258 DVT patients at time point 3 months post-DVT. When in the cohort of 78 patients with rapid recanalization within 90 days (3 months) anticoagulation was discontinued at 3 months post-DVT, the risk of recurrent VTE during

follow-up was 1.3% (95% CI, 1-7%, B in figure 5). The proportion of provoked vs unprovoked DVT was 64% and 36% indicating that this distinction is artificial in terms of risk assessment. The group of 180 DVT patients with residual venous thrombosis (with no or partial recanalization on DUS) were randomized to stop anticoagulation at 3 months post-DVT (N=92) or to continue anticoagulant for 9 additional months and discontinued 12 months post-DVT. During follow-up from the time of anticoagulation discontinuation recurrent VTE events started to occur in 25 of 92 = 15, 2% person-years who discontinued anticoagulation at 3 months post-DVT (A1 in figure) and in 17 of 88 patients = 1.1% person-years who discontinued anticoagulation at 12 months post-DVT (A2 in figure 5). Very interestingly, the proportion of provoked vs unprovoked DVT in patient with RVO (incomplete recanalization) at 3 months post-DVT was 23% vs 77% again indicating that this distinction is artificial in terms of risk on DVT recurrence.

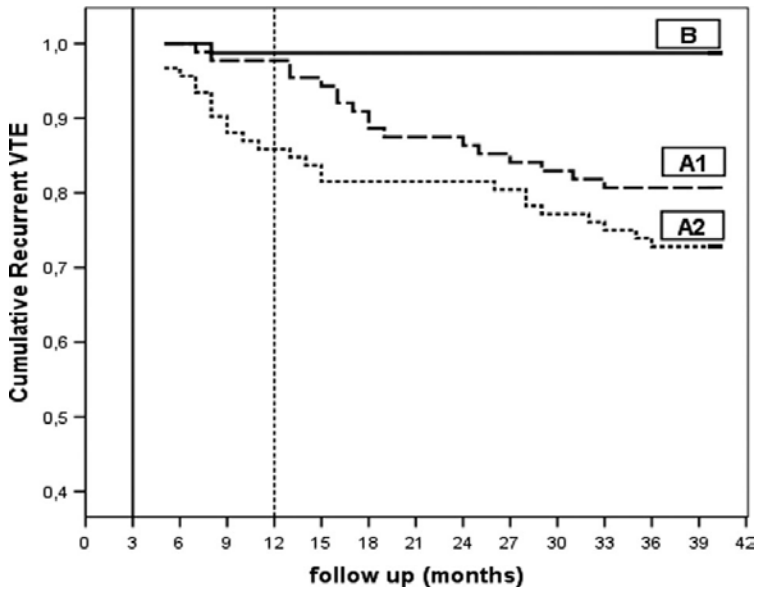

Figure 5 : Discontinuation of anticoagulation at 3 months post-DVT in DVT patients with rapid recanalization of leg veins within 90 days (3 months, N=78) is followed by a very low DVT recurrence rate B). In patients with residual vein occlusion on DUS at 3 months post-DVT discontinuation of anticoagulation at 3 months (A2, N=92) or at 12 months DVT (A1, N=88) is followed by increased VTE recurrence rate[15]

These two prospective studies demonstrate that half of the patients with a first DVT do not need therapy with MECS in cases with rapid complete recanalization and no PTS at 3 months post-DVT obviating the need of anticoagulant treatment at 6 months post-DVT[14,15.] Early mobilisation of the patient, adequate anticoagulant therapy and immediate compression treatment with MECS reduces the chance to develop PTS significantly. In the prospective study of 313 consecutive DVT patients, the cumulative incidence of normal DUS (no RVT) was 39%, 58%, 69% and 74% at 6, 12, 24 and 36 months post DVT respectively. About one quarter of DVT patients had RVO at time point 3 years post-DVT[12]. Of 58 VTE recurrent episodes, 41 occurred at time of RVT. The hazard ratio for recurrent VTE was 2.4 with persistent RVO versus those with earlier complete vein renalization[12]. In view of this, three subsequent yet unanswered questions in the treatment of DVT are[13]:

1. Which DVT patient has a clear indication for long-term compression therapy to prevent PTS after the initial anticoagulant treatment in the acute phase of DVT at time point 6 months post-DVT?
2. Is 3 months post-DVT the appropriate points during DUS follow-up to determine candidates who are at risk to develop PTS?
3. It is unknown what should be done with these patients after 2 years. Is continuing compression therapy after 2 years effective in the prevention of PTS?

VI. Rapid Versus Delayed Recanalization Related to Reflux in Post-DVT Patients Predict PTS

Phlebologists generally distinguish two main types of PTS[13].

1. The reflux type (circa 90%)
2. The obstruction type (circa 10%)

In a substantial number of patients only partial re-canalisation (=residual venous occlusion: RVO) occurs as opposed to complete obstruction or nearly complete obstruction. In the prospective study of Prandoni et al the cumulative incidence of complete recanalization was 39%, 58%, 69%, and 74% at 6, 12,

24 and 36 months (1/2, 1, 2 and 3 years) post DVT respectively[12]. Sequential DUS imaging can easily assess this.

DVT patients with complete re-canalisation (no RVO) can be divided in two groups[3]:

1. Those with functional (intact) vein valves
2. Those with dysfunctional vein valves

Dysfunctional vein valves result in reflux and ultimately in increased venous pressure, which is the main hemodynamic determinant of symptomatic PTS in need for MECS and extended anticoagulation. Asymptomatic post-DVT patients with complete re-canalisation but with no reflux or compensated reflux (low risk DVT recurrence) might be candidates for discontinuation of MECS therapy within the period of 2 years[13].

Meissner *et al* studied the relationship between time to complete recanalization post-DVT (lysis time) and the development of reflux in patients with a first episode of DVT at 3 months interval during the first year (figure 6)[16]. Duplex criteria for complete occlusion were defined as the absence of detectable flow, either spontaneous or with augmentation, in an incompressible venous segment. Partial occlusion was defined as normal or diminished flow, either spontaneous or with augmentation, in an incompletely compressible venous segment. Complete lysis (recanalization) was presumed to have occurred when spontaneous phasic flow returned and the vein was completely compressible[16]. For the posterior tibial veins (PTV), flow detected after distal augmentation in a completely compressible vein is accepted as evidence of complete recanalization (lysis). The median time from DVT to complete recanalization (lysis time) was about 3 months (100 days) for patients without reflux in all segments (figure 3). In contrast, the median time from DVT to complete recanalization (lysis time) of all segments was about 9 to 12 months (more than 6 months) for DVT patients who developed reflux as the main determinant of symptomatic PTS (figure 6). As shown in figure 2, loss of valve competence leading to ambulatory venous hypertension (AVP) and diversion of venous flow through incompetent perforator veins appear to play an important role in the development of late complications of PTS. In the study of 123 legs with DVT (107 patients) by Markel et al about two third of the involved legs had developed valve incompetence (figure 7)[17]. The distribution of reflux at the end of the first year follow-up in this study was the following: popliteal vein, 58%, femoral vein, 37%, greater saphenous vein, 25% and posterior tibial vein, 18%. Reflux appeared to be more frequent in the segments previously affected by DVT (figure 7)[17]. The data of the DVT-LYSIS reflux studies in figures 3 and 4 will predict that discontinuation of anticoagulation at 3 months post-DVT in DVT patients with rapid recanalization of leg veins within 90 days (3 months, N=78 figure 6) will be followed by a very low DVT recurrence rate B). In patients with delayed recanalization at 6 to 12 months post-DVT

DVT discontinuation are at high risk for reflux and PTS development and therefore candidates for continuation of MECS and anticoagulation for at least 1 to 2 years.

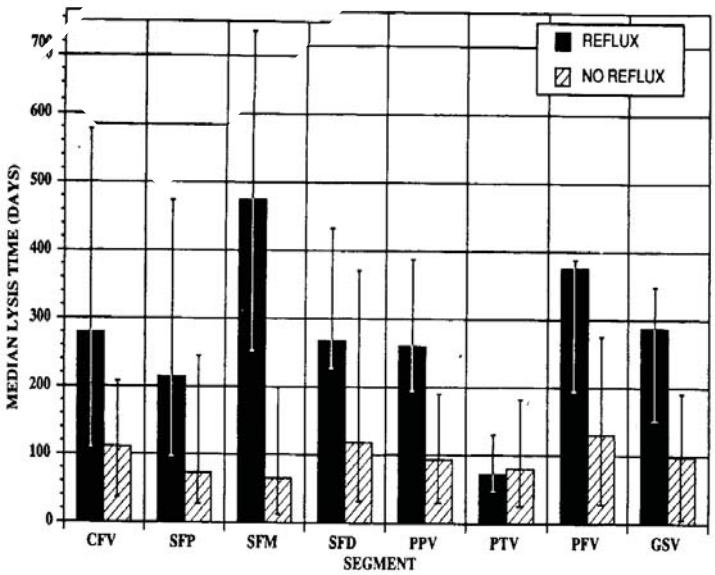

Fig. 1. Median time from thrombosis to recanalization for all segments (lysis time), grouped according to reflux status at last visit. *Error bars* show interquartile range (25th to 75th percentile).

Segment	Reflux n	Reflux Median lysis time (days)	Reflux Interquartile range	No reflux n	No reflux Median lysis time (days)	No reflux Interquartile range	p Value
CFV	10	278	104-577	14	111	37-208	0.14
SFP	15	214	95-474	18	73	30-245	0.086
SFM	10	474	245-736	16	65	15-199	0.002
SFO	7	268	230-433	13	118	34-371	0.15
PPV	10	260	187-388	20	93	29-190	0.006
PTV	8	72	49-130	38	80	28-182	0.85
PFV	5	375	289-388	10	130	30-275	0.04
GSV	6	287	145-348	7	98	4-191	0.11

Figure 6 : The median time from DVT to complete recanalization (lysis time) was about 3 months (100 days) for 118 patients without reflux in all segments. The DVT patients with rapid recanalization had popliteal or distal DVT in 68 of 118 and proximal femoral DVT in 46 of 118. In contrast, the median time from DVT to complete recanalization (lysis time) of all segments was delayed to about 9 to 12 months (more than 6 months) in 65 DVT patients who developed reflux as the main determinant of symptomatic PTS. The majority of DVT patients with delayed recanalization and reflux had proximal femoral DVT in 42 of 52 patients and popliteal DVT in 10 of 52 DVT patients. SFM: middle superficial femoral vein, SFD: distal superficial vein, PPT: popliteal vein, PTV: posterior tibial vein, GSV: greater saphena vein)[16] .

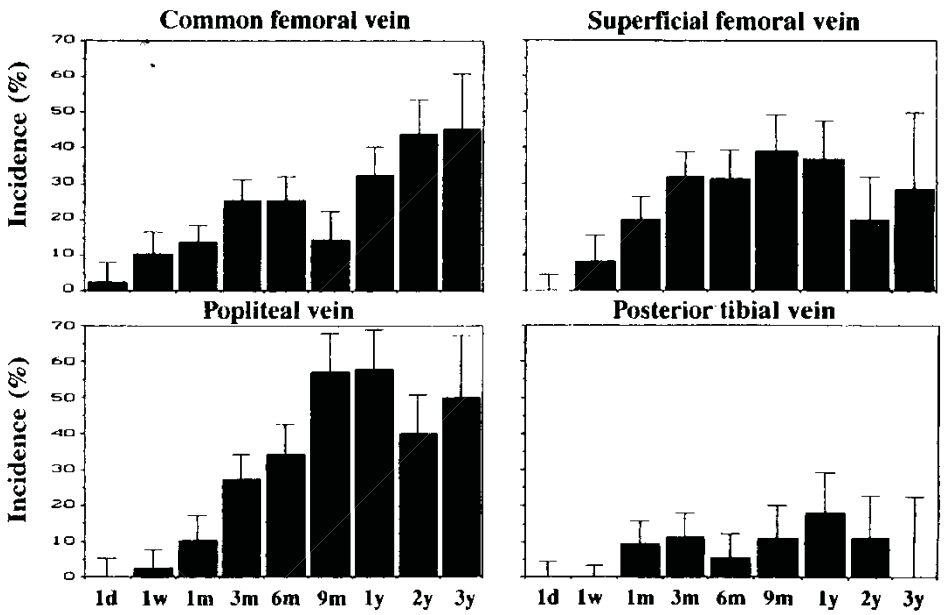

Figure 7 : Incidence of reflux in deep veins at the different follow-up intervals: 1 month, 3 months, 6 months, 9 months, 1 year, 2 years, 3 years in the in the group of post-DVT patients with delayed recanalization of 6 to 12 months in the DVT-LYSIS-Reflux Study[17].

In the Maastricht study[18], 125 consecutive DVT patients with confirmed proximal DVT (22% had a history of previous VTE) were followed for 2 years using the Vilalta clinical scores on four consecutive visits; 3, 6, 12, and 24 months post-DVT. After 6 months, patients with Vilalta scores ≤4 in the absence of reflux on DUS were allowed to discontinue MECS therapy. If reflux was present, two consecutive scores ≤4 were needed to discontinue MECS therapy. Accordingly, MECS therapy was discontinued in 17% of patients at 6 months, in 48% at 12 months, and in 50% at 24 months. Reflux on CUS was present in 74 of 101 (73.3%) tested patients. Reflux alone was not determinative for the prediction of PTS. The cumulative incidence of PTS was 13.3% at 6 months, 17.0% at 12 months, and 21.1% at 24 months. Varicosis or venous insufficiency (present in 11% at baseline) was significantly associated with PTS; hazard ratio 3.2 (1.2-9.1). In this study patients with a low probability for developing PTS can be identified as early as 6 months post-DVT[19,20]. Individualized shortened duration of MECS therapy in post-DVT patients with no or asymptomatic reflux on Vilalta clinical scores seems to be a safe management option.

DUS at 6 months post-DVT in unprovoked DVT and DVT recurrence rate after discontinuation of anticoagulation is not predictive for DVT recurrence.

In a post-hoc analysis in a selected large group of 452 patients with unprovoked DVT (table 7), Le Gal et al performed DUS at time point 6 months if the post-DVT patient had signs or symptoms at time point 5 to 7 months post-DVT when oral anticoagulation (OAT) was stopped19. In the study of Le Gal et al 184 of 452 (46%) had overt PTS with any hyperpigmentation (=CEAP >4), edema (CEAP >3) or redness in either leg (table 3). Recanalizattion on DUS was complete in 220 and abnormal in 231 with minimal wall thickening in 11, partial thrombus resolution in 78 and minimal thrombus resolution (obstruction) in 23 and missing data in 67, but reflux was not measured. During follow-up off OAT 45 of 231 with abnormal CUS (19.5%) and 32 out of 220 patients (14.6%) with normal DUS (complete recanalization) had recurrent VTE (figure 8)[19].

Table 7 : Baseline characteristic of 452 patients with unprovoked DVT at time point 6 months post-DVT at time of discontinuation of anticoagulation[19].

Characteristics	*n* (%) or mean (SD)
Female gender	203 (44.9)
Age, years	54 (17)
Weight, kg	89 (22)
Height, cm	172 (11)
Body mass index, kg m^{-2}	29.5 (6.8)
D-dimer, ng mL^{-1} (Vidas D-dimer)	312 (421)
Any hyperpigmentation, edema or redness in either leg	184 (46)

SD, standard deviation.

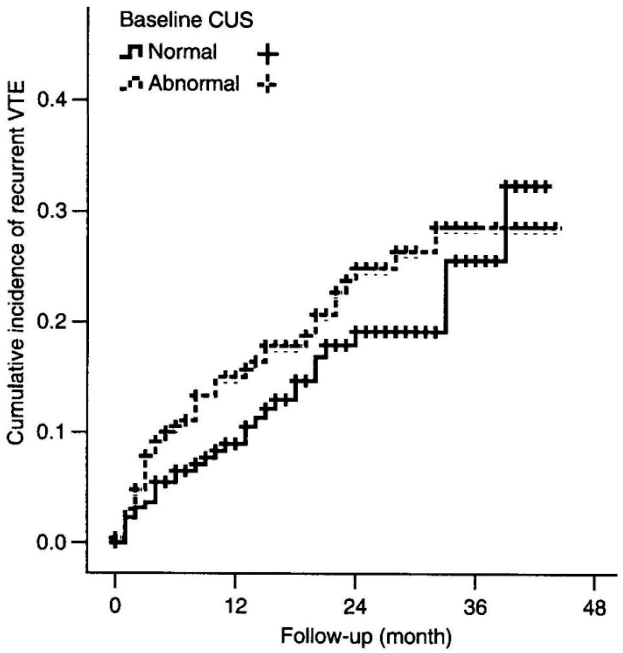

Figure 8 : Cumulativeincidence of recurrent VTE afterdiscontinuation of anticoagulation with baseline DUS evaluation at time point 6 months post-DVT in 231 patients with abnormal CUS (minor, partial or nearly complete recanalization) and in 220 patients with normal DUS (complete recanalization)[19].

Palaretiet al showed that normal versus increased D-dimer levels one month after discontinuation of regular anticoagulation is associated with an incidence DVT recurrence of about 5% patient-years and 10 to 15% patient/years respectively[20]. This difference was independent from other factors like thrombophilia or presence or absence of RVT (residual vein thrombosis). Such post-DVT patients with increased

D-dimer abpve the upper limit of normal after discontinuation surely belong to the group of symptomatic post- DVT patients at high risk to develop PTS. In the prolong study, extended anticoagulationin post-DVT patients with increased D-dimer reduced the risk of DVT recurrence from 11% patient/years to less than 2% patient/years, whereas the incidence of DVT recurrence was still increased, 4.4% patient/years, in post-DVT patients with a normal D-dimer on month after discontinuation of regular anticoagulation[20]. This may implicate that DVT recurrence in those patients with either a normal or increased D-dimer very likely do occur in those with incomplete or complete RVT after 6 months. Reflux was not measured in this study.

As the extension of the PROLONG study Palareti performed the DULCIS (D-dimer and ultrasound in combination Italian Study) to establish the optimal duration of anticoagulation for VTE in 988 evaluable DVT patients with a first unprovoked DVT[21]. After at least 3 months of anticoagulation D-dimer was measured and DUS performed to measure residual venous thrombosis (RVT <4 mm) and followed according two main strategies. First, if the D-dimer level was below age and gender specific cut-off for each of the different D-dimer assay used, antiocoagulation was stopped and D-dimer levels were reassessed at 15, 30, 60 and 90 days. If at time of at least 6 months post-DVT the D-dimer remained below the cut-off, anticoagulation was definitely stopped and patients were followed up for 2 years. In 506 (51%) of the 988 analyzed patients all D-dimer were below the cut-off in the 3 months (90 days) after stopping anticoagulation. The incidence of VTE was 2.8%, distal DVT 1.1% and superficial venous thrombosis (SVT) in 2.3% patient/years. Second, if one of the D-dimer levels was above the cut-off in the period of 3 month (90 days) after discontinuation anticoagulation was resumed. This cohort 373 patients with increased D-dimer levels above the age and gender adjusted cut-off levels received oral anticoagulation for 2 years follow-up and only 4 VTE events (0.7% patient/years) were observed at the cost of 14 major bleedings (2.3% patients/years)[21]. In a third cohort 109 patients (11% patient/years) with at least one D-dimer measurement above cut-off, who refused oral anticoagulation treatment the incidence of major VTE was 8.8%, and distal DVT or SVT 2.3% patient/years.

VII. DVT and PTS Bridging the Gap

In view of the proposed concept in figure 6, complete recanalization within 1 to 3 months and no reflux is predicted to be associated with no PTS obviating the need of anticoagulation and MECS at 6 months post-DVT. Delayed incomplete or complete recanalization at 6 to 12 months post-DVT is associated with a high risk of DVY recurrence as the cause of PTS and usually complicated by reflux due to valve destruction, thereby indicating the need to extend anticoagulant treatment and wearing MECS. Patients with delayed but complete recanalization with reflux or without RVT but no PTS at 6 to 12 months post-DVT while wearing MECS are candidates for extended anticoagulation for about one year and to be re-evaluated at time point one year post-DVT according to the PROLONG and DULCIS strategy (figures 9 and 10)[13,20,21]. Patients with PTS according to subjective Villalta and/or CEAP score at 6 to 12 months post-DVT should be anticoagulated for 2 years and are to be considered for extended or even life-long anticoagulation with VKA or NOAC[13]. As prevention of DVT recurrence as the best option to prevent the occurrence and progression of PTS. Michiels &Moosdorff started in 2012 a pilot study to test the feasibility of a multicenter prospective management and safety outcome study to further improve the proposed concept in figures 9 and 10 in the primary care setting.

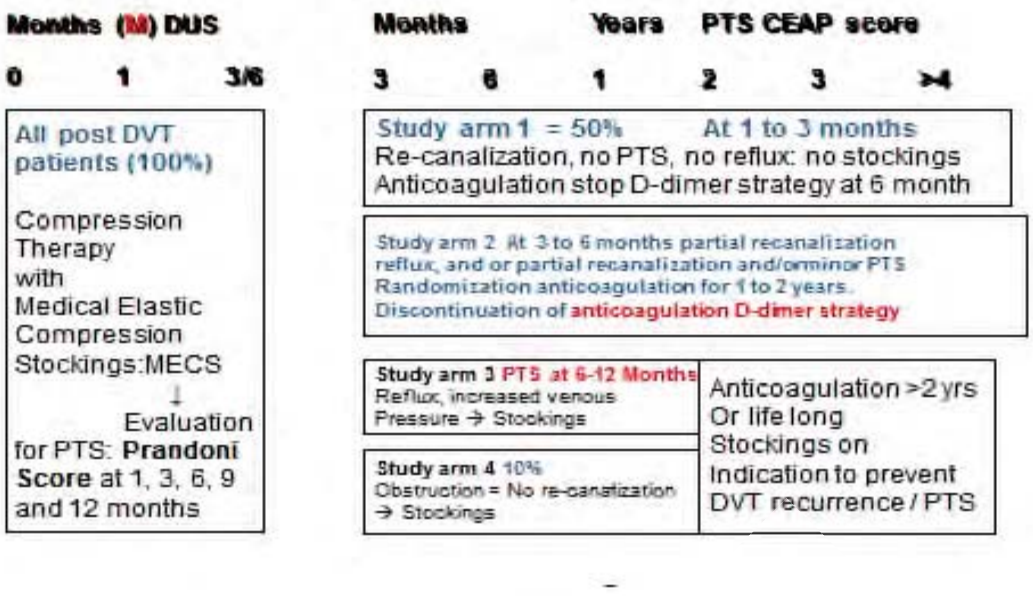

Figure 9 : Erasmus Study design to bridge the gap between DVT and PTS related to the indication of wearing MECS and the duration of anticoagulation with VKA or NOAC according to Michiels, Moosdorff& Neumann[13].

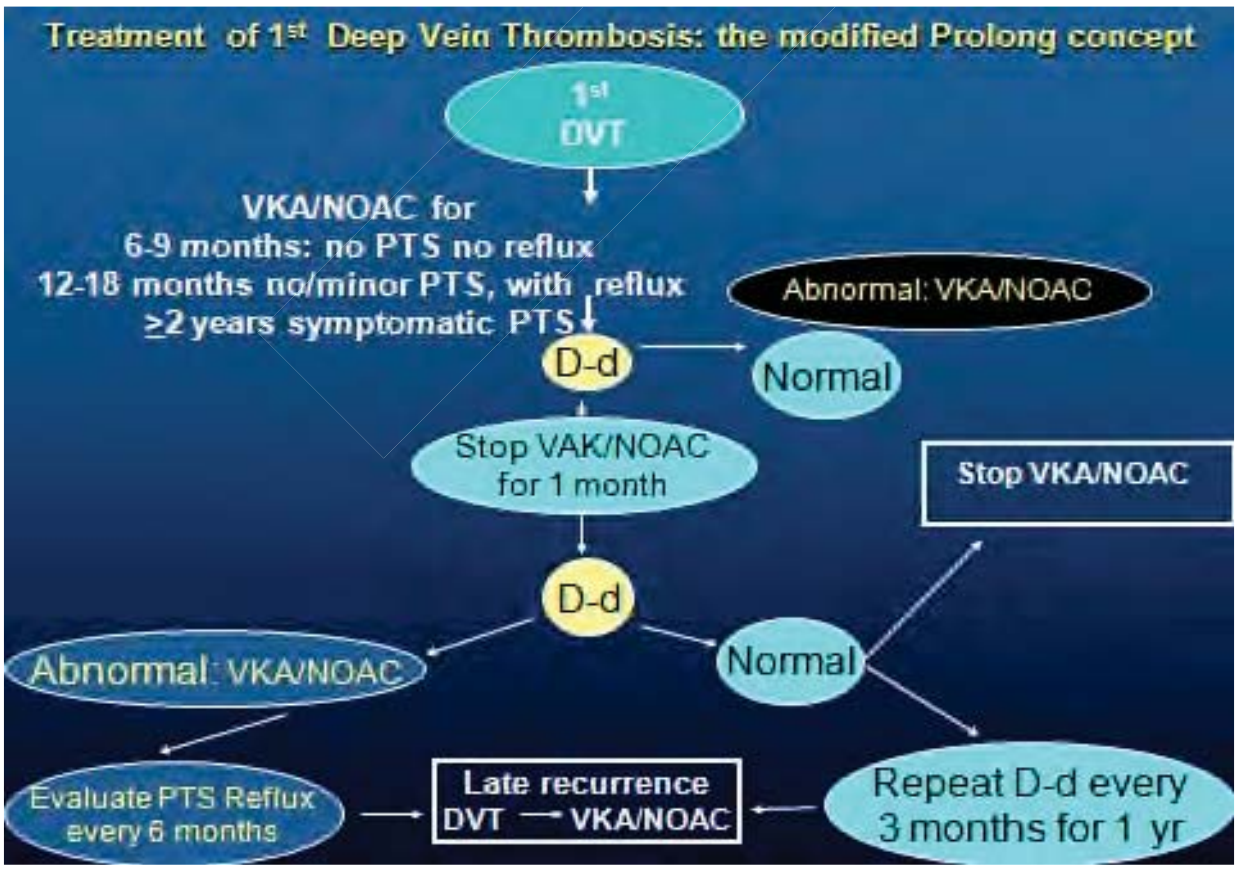

Figure 10 : Algorithm according to the Rotterdam modification of the PROLONG study for anticoagulant treatment with vitamin K antagonist (VKA) or novel oral anticoagulants (NOAC) in post-DVT patients to prevent DVT recurrence as the cause or progression of PTS[13,20,21].

References Références Referencias

1. Michiels JJ. Section Thrombophilia in: Thrombophilia and Venous Thromboembolism. International Consensus statement. Guidelines according to scientific evidence. Int Angiol 2005;24:1-26. .
2. Tick LW, Kramer MHH, Rosendaal FR, Fabers WR, Doggens CJM. Risk factors for post-thrombotic syndrome in patients with a first deep venous thrombosis. J ThrombHaemostas 2008;6:2075-2081.
3. Michiels JJ, Hamulyak K. Laboratory diagnosis of hereditarythrombophilia. Sem ThrombHemostas 1998;24:309-320.
4. Michiels JJ, Gadisseur A, van der Planken M, Schroyens W, de Maeseneer M et al.Screening for deep vein thrombosis and pulmonary embolism in outpatients with suspected DVT or PE by sequential use of clinical score: A sensitive quantitative D-dimer test and non-invasive diagnostic tools. *Sem in Vasc Med* 2005;5:351-64.
5. Michiels JJ, Gadisseur A, van der Planken M, Schroyens W, de Maeseneer M *et al*.A critical appraisal of non-invasive diagnosis and exclusion of deep vein thrombosis and pulmonary embolism in outpatients with suspected deep vein thrombosis or pulmonary embolism: how many tests do we need? *Int Angiol* 2005;24:27-39.
6. Kearon C, Ginsberg JS, Douketis J, Crowther MA, Turpie AG, Bates SM et al. A randomized trial of diagnostic strategies after normal proximal vein ultrasonography for suspected deep vein thrombosis: D-Dimer testing compared with repeated ultrasonography. Ann Intern Med 2005;142:490-496.
7. Perrier A, Desmarais S, Miron MJ, De moerloose P, Lepage R, Slosman D, Didier D, Unger PF, Patenaude JV, Bounameaux H. Non-onvasive diagnosis of venous thromboembolism in outpatients. Lancet 1999;353:190-195.
8. Schutgens RE, Ackermark P, Haas FJ, Nieuwenhuis HK, Peltenburg HG, Pijlman AH, Pruijm M, Oltmans R, Kelder JC, Biesma DH. Combination of a normal d-dimer concentration and a non-high pretest clinical probability score is a safe strategy to exclude deep vein thrombosis. Circulation 2003;107:593-597.
9. Oudega R, Toll DB, Bulten RJ, Hoes AW,Moons KG. Different cut-off levels for two D-Dimer assays to exclude deep vein thrombosis in primary care. ThrombHaemostas 2006;95:744-746.
10. Michiels JJ, Moosdorff W, Maasland H, Michiels JM, Lao MU, Neumann HAM, Dulicek P, Stvrtinova V, Barth J, Palareti G. Duplex ultrasonography, clinical score, thrombotic risk and D-dimertesting for evidence baseddiagnosis and management of deepve in thrombosis and alternative diagnoses in the primary care setting and out patientward. Intern Angiol 2014;33(1):1-19.
11. Prandoni P, Lensing AWA, Cogo A et al. The long-term clinical course of acute deep vein thrombosis. Ann Intern Med 1996;125:1-7.
12. Prandoni P, Lensing AWA, Prins MH et al Residual venous thrombosis as a predictive factor of recurrent venous thromboembolism. Ann Intern Med 2002;137:955-960.
13. Michiels JJ, Moosdorff W, Maasland H, Michiels JM, Lao MU. Neumann HAM, Dulicek P, Stvrtinova V, Palareti G. Prospective Studies on Diagnosis, Prevention, and Management of DeepVeinThrombosis (DVT), DVT Recurrence and the Post-ThromboticSyndrome(PTS): From Concept toStudy Design in the Primary Care Setting. Fam Med MedSciRes 2014;(3):138
14. Prandoni P, Lensing A, Prins MH et al. Below-knee elastic compression stockings to prevent the post-thrombotic syndrome. Ann Int Med 2004;141:249-56.
15. Siragusa S, MalatoA, Anastasio R et al. Residual vein thrombosis to establish duration of antiocoagulation after a first episode of deep vein thrombosis. Blood, 2008;112:511-515.
16. Meissner MH, Manzo RA, Bergelin RO, Markel A, Strandness E. Deep venous insufficiency: the relationship between lysis and subsequent reflux. J Vasc Surgery 1993;18:596-608.
17. Markel A, Manzo RA, Bergelin RO, Strandness DE. Valular reflux after deep vein thrombosis: incidence and time of occurrence. J VascSurg 1992;15:377-384.
18. Ten Cate-Hoek AJ, TenCate H, Tordoir J, Hamulyak K, Prins MH. Individually tailored duration of elastic compression therapy in relation to incidence of the post-thrombotic syndrome. J VascSurg 2010;52:132-138.
19. Le Gal Carrier M, Kovacs MJ, Betancourt SMT, Kahn SR, Wells PS, Anderson DA, Chagon J, Solymoss S, Crowther M, Righini M, Delluc A, White RH, Vickars L, Rodger M. Residual vein obstruction as a predictor for recurrent thromboembolic events after a first unproved episode: data from the REVERSE study. J ThrombHaemostas 2011;9:1126-1132.
20. Palareti G, Cosmi B, Legnani C et al. D-dimer testing to determine the duration of anticoagulant therapy. New Eng J Med 2006;355:1780-1789.
21. Palareti G, Cosmi B, Legnani C, Antonucci E, De Micheli V, et al. D-dimerto guide the duration of anticoagulation in patients with venous throm boembolism: a management study. Blood 2014;124: 196-203.

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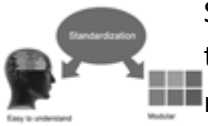
As FARSM, you will be given a renowned, secure and free professional email addres with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.



The FARSM will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSM member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSM, you may send us a scanned copy of all of you credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

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The ' MARSM ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The "MARSM" is a dignified ornament which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., MARSM or William Walldroff, M.S., MARSM.

MARSM accrediting is an honor. It authenticates your research activities. Afterbecoming MARSM,you can add 'MARSM' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

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As MARSM, you willbe given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSM member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSM, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

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- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone.

"

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

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- Paper Title should be of Font Size 24 with one Column section.
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- Two Column with Equal Column with of 3.38 and Gaping of .2
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You can use your own standard format also.
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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

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Complete support for both authors and co-author is provided.

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Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

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(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

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It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

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All manuscripts submitted to Global Journals Inc. (US), ought to include:

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Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

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Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

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Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

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Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

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TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

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3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

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5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

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- Do not give permission to anyone else to "PROOFREAD" your manuscript.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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