ISSN 2307-8960 (online)

World Journal of *Clinical Cases*

World J Clin Cases 2017 May 16; 5(5): 159-190

Published by Baishideng Publishing Group Inc

WJCC

World Journal of Clinical Cases

A peer-reviewed, online, open-access journal of Clinical Cases

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2017-2020

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World Journal of Clinical Cases

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Monthly Volume 5 Number 5 May 16, 2017

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World Journal of Clinical Cases
Volume 5 Number 5 May 16, 2017

NAME OF JOURNAL
World Journal of Clinical Cases

ISSN
ISSN 2307-8960 (online)

LAUNCH DATE
April 16, 2013

FREQUENCY
Monthly

EDITORIAL OFFICE
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World Journal of Clinical Cases
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
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E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
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PUBLISHER
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7901 Stoneridge Drive,
Suite 501, Pleasanton, CA 94588, USA
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Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
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http://www.wjgnet.com

PUBLICATION DATE
May 16, 2017

INSTRUCTIONS TO AUTHORS
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ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Clinical Cases

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World J Clin Cases 2017 May 16; 5(5): 159-171

DOI: 10.12998/wjcc.v5.i5.159

ISSN 2307-8960 (online)

REVIEW

Evolution, current status and advances in application of platelet concentrate in periodontics and implantology

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Author contributions: Agrawal AA contributed all to this paper.

Conflict-of-interest statement: Agrawal AA declares no conflict of interest related to this publication.

Manuscript source: Invited manuscript

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Received: September 12, 2016
Peer-review started: September 13, 2016
First decision: October 21, 2016
Revised: January 29, 2017
Accepted: February 28, 2017
Article in press: March 2, 2017
Published online: May 16, 2017

Abstract

Platelet concentrates (PC) [platelet-rich plasma (PRP) and platelet-rich fibrin (PRF)] are frequently used for surgical procedures in medical and dental fields, particularly in oral and maxillofacial surgery, plastic surgery and sports medicine. The objective of all these technologies is to extract all the elements from a blood sample that could be used to improve healing and promote tissue regeneration. Although leukocyte rich and leukocyte poor PRP's have their own place in literature, the importance of non-platelet components in a platelet concentrate remains a mystery. PC have come a long way since its first appearance in 1954 to the T-PRF, A-PRF and i-PRF introduced recently. These PC find varied applications successfully in periodontics and implant dentistry as well. However, the technique of preparation, standing time, transfer process, temperature of centrifuge, vibration, *etc*., are the various factors for the mixed results reported in the literature. Until the introduction of a proper classification of terminologies, the PC were known by different names in different countries and by different commercial companies which also created a lot of confusion. This review intends to clarify all these confusion by briefing the exact evolution of PC, their preparation techniques, recent advances and their various clinical and technical aspects and applications.

Key words: Platelet concentrates; Platelet rich plasma; Platelet-rich fibrin; Pure-platelet-rich fibrin; Leukocyte- and platelet-rich fibrin; Sticky bone; Platelet derived growth factors; Fibrin glue

Core tip: Platelets concentrates are known to be a rich source of growth factors with added antimicrobial efficacy due to incorporations of leukocytes. But does that mean that platelets or platelet poor/depleted plasma do not have any antimicrobial role? Are the mixed results reported in the literature due to deviations from the manufacturing protocols and nomenclature of platelet concentrates (PC)? Does technical factors related to centrifuge speed, time, temperature, vibrations, resonance, *etc.*, affect the biological quality of the resultant platelet concentrate? A thorough knowledge evolution, preparation and applications of various PC will help clinicians to use this arsenal more efficiently.

Agrawal AA. Evolution, current status and advances in application of platelet concentrate in periodontics and implantology. *World J Clin Cases* 2017; 5(5): 159-171 Available from: URL: http://www.wjgnet.com/2307-8960/full/v5/i5/159.htm DOI: http://dx.doi.org/10.12998/wjcc.v5.i5.159

INTRODUCTION

An average baseline platelet count in humans is 200000 ± 75000/μL with a half-life of 7-10 d. Platelets are irregularly shaped, small (2-4 μm) anuclear cells, derived from fragmentation of precursor megakaryocytes. They contain few mitochondria, many granules and 2 prominent membrane structures, the dense tubular system and the surface connected canalicular system. Activated platelets trigger their major effects by substances located in one of the three different types of platelet granules: A-granules, dense granules, and lysosomes. Alpha granules are the most abundant type and contain many different bioactive mediators. They are spherical or oval structures (200 to 500 nm), enclosed by a unit membrane. Upon contact with exposed endothelium (due to damage tissue or wound) the platelets get activated and are known to release key wound healing factors: Platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF) and epidermal growth factor (EGF). Platelets begin to actively secrete these proteins within 10 min after clotting, with more than 95% of the pre synthesized growth factor secreted within 1 h. For the balance of their life (5 to 10 d), the platelets synthesize and secrete additional proteins. As the direct platelet influence begins to subside, macrophages, which arrive by means of vascular ingrowth stimulated by the platelets, assume responsibility for wound-healing regulation by secreting their own factors. Thus, the platelets at the repair site ultimately set the pace for wound repair.

Platelet concentrates (PC) [platelet-rich plasma (PRP) and platelet-rich fibrin (PRF)] are frequently used for surgical procedures in many medical fields[1], particularly in oral and maxillofacial surgery[2,3], plastic surgery[4] and sports medicine[5,6]. The objective of all these technologies is to extract (through centrifugation) all the elements from a blood sample that could be useful to improve healing and promote tissue regeneration[7], particularly: The platelets (rich in growth factors)[8], the fibrin (supporting matrix)[9] and in some cases the cell content (mostly leukocytes)[9]. A natural blood clot contains 95% red blood cells, 5% platelets, less than 1% white blood cells, and numerous amounts of fibrin strands. A PRP blood clot contains 4% red blood cells, 95% platelets, and 1% white blood cells. The literature on these products is quite confusing and controversial due to the lack of proper characterization of these many different products[10,11]. Compared to application of single, supra-physiological concentrations of recombinant growth factors, PC has the advantage of offering multiple, synergistically working growth factors at the wound site and in concentrations that are physiologically and biologically more relevant. But the question is whether it is only the platelet in PC's that plays lead role or are the non-platelet components equally important when considering the clinical applications. Some authors have in-fact suggested that RBC's and WBC's could be pernicious as they may contribute in inflammatory reactions leading to damage of the treated tissues[12-14]. Until these controversies are resolved in clinical literature, a big question still persists whether the non-platelet cellular components of PC have any role in their biological activities such as platelet activation and subsequent release of growth factors?

The natural healing process in any wound starts as blood coagulation leading to fibrin/platelet clot and matrix. PC's were introduced to reinforce this natural wound healing process. For example fibrin glues which are being used as surgical adjuvants since > 40 years. Over the period, this idea evolved to a more refined concept of tissue regeneration which was enhanced by the cells and the growth factors contained in these preparations. Initially used as surgical adjuvant, the PRP/PRF became the new glorified regenerative medicine approach. Platelets, leukocytes, fibrin, growth factors and other cells are the primary active players in the physiological wound healing process. Combined together they form a kind of engineered tissue which is derived from the blood circulation. However, this complex combination is ultimately decisive for the optimal performance. Therefore, the L-PRF clot, *i.e.*, Leukocyte-and PRF, was commonly known as an "optimized blood clot".

EVOLUTION OF PC

1954

Kingsley[15] first used the term PRP to earmark thrombocyte concentrate during experiments related to blood coagulation.

1970

"Fibrin glue" was introduced by Matras[16] which improved healing of skin wounds in rat models. Fibrin glue was made by polymerizing fibrinogen with thrombin and calcium. However, due to low concentration of fibrinogen in donor plasma, the quality and stability of fibrin glue was suboptimal.

1975-1978

Numerous research works suggested an enhanced concept for using blood extracts and designated them as "platelet-fibrinogen-thrombin mixtures"[17].

1979

Another author called it "gelatin platelet - gel foam". This new proposition asserted the performance of platelets, and demonstrated exquisite preliminary results in general surgery, neurosurgery and ophthalmology. However till then all these products were used primarily for their "gluey

effect", without consideration of effects of growth factors or their healing properties.

1986

Knighton *et al*[18] first demonstrated that PC successfully promote healing and they termed it as "platelet-derived wound healing factors (PDWHF)", which was successfully tested for the management of skin ulcers.

1988, 1990

Kingsley *et al*[15] and Knighton *et al*[19] used a slightly different term "platelet-derived wound healing formula (PDWHF)".

1997

Whitman *et al*[20] named their product PRP during preparation but when the end product had a consistency of a fibrin gel and therefore labeled it as "platelet gel".

1998

The development of these techniques continued slowly until the article of Marx *et al*[21], which started the craze for these techniques. However, all these products were designated as PRP without deliberation of their content or architecture, and this paucity of terminology continued for many years. Some commercial companies, in lieu of better visibility, started labeling their products with distinct commercial names.

1999

One of the popular methods advertised on large scale to prepare pure platelet rich plasma was commercialized as plasma rich in growth factors (PRGF) or also called as preperation rich in growth factors (Endoret, Victoria, Biotechnology Institute BTI, Spain). However, because of lack of specific pipetting steps and also lack of ergonomics, there were significant issues with this technique[11]. Another widely promoted technique for P-PRP was commercialized by the name Vivostat PRF (Alleroed, Denmark). However, as the name implies it is not a PRF but produces a PRP product.

2000

Simultaneously, Choukroun *et al*[22] developed another form of PC in France which was labeled as PRF, based on the strong fibrin gel polymerization found in this preparation. It was stamped as a "second-generation" platelet concentrate because it was obviously different from other PRPs. This proved an important milestone in the evolution of terminology.

2006

Bielecki *et al*[23] and Cieslik-Bielecka *et al*[24,25] proposed to define PRP as inactive substance, while PRG (Platelet Rich Gel) was a more biologically activated fibrin matrix rich in platelets, leukocytes and relative active molecule.

Sacco[26] introduced a new concept of CGF (concentrated growth factors). For making CGF from venous blood, rpm in range of 2400-2700 was used to separate cells. The fibrin rich blocks that were obtained were much larger, richer and denser.

2008

Everts *et al*[27,28] focused on the leukocyte component of the platelet concentrate and the two forms, *i.e.*, non-activated and activated. The inactivated/non-activated product was called "platelet-leukocyte rich plasma (P-LRP) and activated gel was labeled platelet-leukocyte-gel" (PLG).

2009

The first classification about platelet concentrate was proposed by Dohan Ehrenfest *et al*[11]. This classification defined 4 main families based on separation of the products using 2 key parameters: The cellular content (primarily leukocytes) and the fibrin architecture: (1) Pure platelet-rich plasma (P-PRP) - or leukocyte-poor platelet-rich plasma (LP-PRP); (2) Leukocyte-and platelet-rich plasma (L-PRP); (3) Pure PRF (P-PRF) - or leukocyte-poor PRF; and (4) Leukocyte- and platelet-rich fibrin (L-PRF).

2010

Concept of sticky bone (autologous fibrin glue mixed with bone graft) was introduced by Sohn[29] in 2010.

2012

Mishra *et al*[30] proposed another classification which was limited to PRP and applicable to sports medicine only. They stated 4 types of PRP based on presence or absence of leukocytes and whether or not the PRP is activated and all types can fall into 2 sub-types: A: Platelets > 5 × baseline or B: Platelets < 5 × baseline. In all the following types "solution" means non-activated PRP and gel means activated PRP. Type 1: L-PRP solution; Type 2: L-PRP gel; Type 3: P-PRP solution; Type 4: P-PRP gel.

At about the same time DeLong *et al*[31] introduced another classification system called PAW (Platelets quantity, Activation mode, White cells presence). However it again was only restricted to PRP families and was similar to classification by Mishra *et al*[30].

2014

Choukroun[32] introduced an advanced PRF called A-PRF (claimed to contain more monocytes). Tunalı *et al*[33] introduced a new product called T-PRF (Titanium-prepared PRF).

2015

Mourão *et al*[34] gave detailed technical note on preparation of i-PRF.

EVOLUTION IN PREPARATION TECHNIQUES

Fibrin glues, fibrin sealants or fibrin tissue adhesives are derivatives of human plasma that resemble the final stages

of blood coagulation wherein a fibrin clot is formed, available commercially in Europe since late 1970's. There are two types of fibrin sealants: Homologous and autologous. Homologous/commercial variant was prepared by mixing 2 components, *i.e.*, fibrinogen component containing factor XIII and the thrombin component containing calcium ions. Homologous fibrinogen concentrates were prepared from plasma cryoprecipitate or from Cohn fraction I. However, due to the risk of transmitting infections, later fibrin sealants were prepared from autogenous whole plasma and polymerization was instituted using bovine thrombin.

True concentrate of platelets, was termed PRP, which can be manufactured by using two techniques. Both these techniques differ in their technical aspects: (1) General-purpose cell separators; and (2) Platelet-concentrating cell separators.

The former technique (general-purpose cell separators) requires about 450 mL of blood and also usually requires a hospital setting. Blood is drawn into a citrate-phosphate-dextrose anticoagulant containing collection bag. In the first cycle it is centrifuged at 5600 rpm to separate RBCs, platelet poor plasma (PPP) and PRP. Subsequently the speed of the centrifuge is reduced to 2400 rpm to get a final separation of about 30 mL of PRP from the RBCs. A major advantage of this technique is that the remaining PPP and RBCs can be restituted to the patient's circulation or can be discarded. The ELMD-500 (Medtronic Electromedic, Auto Transfusion System, Parker, CO, United States) cell separator is widely used for this technique. The second technique, Platelet-concentrating cell separators, is more widely used since this equipment can be accommodated in a dental clinic setup. This technology permits the procurement of PRP using smaller quantities of blood. Currently, two such systems are approved by FDA and commercially available: Harvest SmartPrep Platelet Concentrate System (HSPCS; Harvest Technologies, Plymouth, MA, United States) and the 3i Platelet Concentrate Collection System (3i PCCS; 3i Implant Innovations, Palm Beach Gardens, FL, United States). Several studies have been performed to compare the efficacy of these systems[6-8]. Although, traditionally a double-spin technique has been used, authors such as Eby[35] have proposed the use of a single spin technique. The preparation and processing of PRP is quite similar in most of the platelet-concentrating systems, however the anticoagulant used and the speed and duration of centrifugation may differ.

An important evolution of terminology appeared when several authors, particularly the groups of Dohan Ehrenfest *et al*[8,36] pointed out that the PC were also associated with various forms of circulating cells, particularly leukocytes, and labeled it as L-PRP (Leukocyte rich platelet rich plasma). Large number of commercial or experimental systems exists for the preparation of L-PRP. In past years many automated protocols have been developed that require minimum handling of blood products, for example Biomet GPS III (Biomet Inc., Warsaw, IN, United States) and Harvest Smart-PreP (Harvest Technologies, Plymouth, MA, United States). There are also other kits which require more handling of blood products, for example Regen PRP (RegenLab, Le Mont-sur-Lausanne, Switzerland) or Plateltex (Prague, Czech Republic). Rutkowski *et al*[37] (2008) demonstrated single spin centrifugation for 10 min at 1350 g for preparation of PRP and they reported six-times enrichment of platelet concentrate. Interestingly they also reported that platelet morphology changes over a period of 6 h bench set time. In fact, even after 2 h the platelets in PRP started to appear less normal. They concluded that PRP bench set times should not exceed 2 h to maintain maximal levels of growth factors, TGFb1 and of platelet morphology. Akhundov *et al*[38] (2012) claimed to introduce a cost effective technique for procuring PRP. They harvested patient's blood using syringe/Vacutainer tubes containing 10 mmol/L 3.8% citrate. This citrate treated blood was transferred to 50 mL Falcon centrifuge tube and centrifuged for 15 min at 280 g at room temperature. After centrifuge, platelets and plasma were removed using 5 mL pipette and transferred to a new 50 mL Falcon centrifuge tube and centrifuged again for 15 min at 280 g at room temperature. The pellet with 1-2 mL of plasma was transferred to new syringe for use in patient for injection or topical application.

Fukaya *et al*[39] (2014) reported an innovative yet economic technique for preparing PRP which consisted of a modification of a(disposable) 5-mL syringe that was inserted into a regular centrifuge. The syringe was positioned in the centrifuge in such a manner that the platelet rich plasma separated adjacent to the tip of the syringe. They also highlighted that instead of heparin or EDTA (ethylene diamine tetra acetic acid), majority of commercial kits adopt dextrose solution A (ACD-A) as an anticoagulant. Even though coagulation and platelet aggregation are very different and anticoagulants never suppress platelet aggregation, no commercial kits consider adding platelet aggregation inhibitor. It's known that aggregated platelets attach to the wall of syringes and are unable to detach from them easily. However their primary aggregation is reversible and the platelets detach from the syringe wall and float in the plasma again after many hours. But in routine clinical practice we cannot wait so long. Therefore authors have suggested addition of platelet aggregation inhibitor "prostaglandin E1 (PGE1)" to anticoagulant ACD-A for preparation of PRP with dense PDGF-BB.

The sole product in the family of P-PRF is the fibrinet PRFM (Platelet-Rich Fibrin Matrix, Cascade Medical, NJ, United States). These are high-density fibrin network preparation with poor leukocyte content. They exist purely in a strongly activated gel form that cannot be injected or used like conventional fibrin glues but instead can be manipulated like a real solid material for other applications. However an important disadvantage of this technique is its high cost and relative complexity of the procedure as compared to the other forms of PRF available such as the L-PRF. The L-PRF was developed and evaluated as a one-step centrifugation without anti-coagulation or blood activator[40]. However, currently the sole commercially available, FDA approved system for making L-PRF, is the

Intra-Spin L-PRF (Intra-Lock Inc., FL, United States). It has something called "Xpression preparation box", which allows the production of generous quantities of membranes and fibrin in relatively small time. Mazzucco *et al*[41] (2016) compared the mechanical properties of PRF against PRGF and found that the former was stronger. It should be noted that the early protocol to produce L-PRF was 3000 rpm/10 min, while since many years the 2700 rpm/12 min protocol is mostly used that gives much better polymerized L-PRF and therefore stronger membranes than the 3000 rpm/10 min protocol. The original L-PRF system now exists only in one CE/FDA cleared form that is termed Intra-Spin L-PRF as stated above. A brief compilation of different types and techniques of platelet concentrate is presented in Table 1[22,26,29,32-34,41-50].

RECENT ADVANCES

After PRF a concept of "Concentrated Growth Factors (CGF)" was introduced in 2006 by Sacco[26]. A special centrifuge called Medifuge (Italy), is used to prepare CGF, similar to PRF, but with a different centrifugation speed which allows the separation of a fibrin matrix which is much denser, larger and richer in growth factors. CGF has been shown to have a greater versatility and better regenerative capacity, as reported for alveolar ridge and sinus augmentation (Sohn *et al*[51], 2009). In a study, Rodella *et al*[52] could demonstrate the presence of VEGF and TGF-b1in RBC and CGF layers. This suggests that improved CGF procedure could enhance the quantity of growth factors in the CGF layer or, alternatively, a possible use of RBC layer in clinical applications. In addition to this, the existence of CD34 positive cells, within the CGF network, could lead to investigation of their clinical implications in future.

Ample evidence has emerged recently on the role of monocytes on the vessels growth and bone regeneration. Monocytes play an important role in vascularization, bone growth and production of VEGF. Monocytes are known to have BMP receptors and recently it was discovered that they produce BMP-2. In an attempt to incorporate the monocytes within the PRF, Choukroun[32] introduced an advanced PRF called A-PRF™. They have discovered earlier soft tissue growth, more release of BMPs, greater and faster vascularization and more cytokine release than conventional PRF.

A concept of fabricating growth factors-enriched bone graft matrix (also known as "sticky bone") using autologous fibrin glue has been demonstrated since 2010[29]. Sticky bone provides stabilization of bone graft in the defect, and therefore, accelerates tissue healing and minimizes bone loss during healing period. To obtain autologous fibrin glue, 20-60 CC of venous blood is centrifuged at 2400-2700 rpm using a specific centrifuge (Medifuge, Silfradentsrl, Sofia, Italy) for 2 min. Out of the two layers obtained, the deeper layer is RBC's and the superficial layer is AFG. This AFG is then extracted using a syringe and mixed with particulate bone powder and allowed to rest for 5-10 min for polymerization, which results in a yellow colored mass called "sticky bone"[53]. Sohn *et al*[53] also noted that the polymerization can be accelerated by adding the exudates obtained after compression that they used to make CGF membrane. These exudates contained growth factors and autologous thrombin in RBC layer due to which the auto-polymerization completed faster[53]. The resultant sticky bone is moldable, prevents micro and macro movement of grafted bone, entraps platelets and leukocytes in its fibrin network, is natural and prevents ingrowth of soft tissues in graft.

Mourão *et al*[34] (2015) described a technique to obtain an injectable form of PRF called i-PRF. In this technique a short centrifuge for 2 min at 3300 rpm gave an orange color fluid which can be injected or mixed with bone graft to give a well agglutinated "steak" for bone grafting.

Although successful procedures have been reported extensively using Choukran's L-PRF, physicians such as O' Connell[54] had raised concern regarding possible health hazard with the particles of silica in the glass tubes. In spite of the fact that the silica particles are sufficiently dense so as to sediment along with the RBC's, they are small enough so that a fraction of them will remain colloidally suspended in the platelet-poor plasma layers, buffy coat and fibrin and might eventually reach the patient during treatment. In this context a study was done by Dohan Ehrenfest *et al*[9] in 2010 evaluating the cell composition and 3D organization of L-PRF persuaded by different types of collection tubes (such as glass-coated plastic tubes or dry glass) and compression techniques (soft or forcible) on the final L-PRF-membrane architecture. Authors demonstrated that there was no influence of the type of tested tube on the architecture of this second generation PC. However Tunalı *et al*[33] in 2014, introduced a new product called T-PRF (Titanium-prepared PRF). The use of titanium tubes for collection and centrifugation instead of glass tubes was established on the hypothesis that titanium may be a more efficient platelet activator than silica, for preparing L-PRF. Based on light, scanning electron and fluorescence microscopy analysis, Tunalı *et al*[33] concluded that T-PRF has immensely organized network along with a continuous integrity and even the fibrin network was thicker and also it covered larger area.

Anitua *et al*[55] (2015) in an *in-vitro* study, evaluated the outcome of different ozone treatments on biologic properties of PRGF. They found that using "continuous flow protocol" of ozone treatment of PRGF, fibrin scaffold formation, growth factor levels along with proliferative potential was drastically reduced. In contrast, ozone treatment using "syringe method" had no effect on the biological outcomes of this autologous therapy, so ozone therapy in combination with PRGF can be effectively used.

APPLICATION OF PC IN PERIODONTICS AND IMPLANT DENTISTRY

Various *in vitro* studies have demonstrated that PRP exerts

Table 1 Compilation of different platelet concentrates, their discovery and different protocols available

Platelet concentrate type	Method (automated/manual)	Highlights
P-PRP	Cell separator PRP (Automated) Weibrich *et al*[50]	PRP collected by discontinuous method where patient is connected to machine continuously, around 300 mL PRP can be collected. When PRP is obtained from a blood bag of 450 mL, 40 mL of PRP can be obtained per bag. Differential ultracentrifugation employed (3000 g)
	Vivostat PRF (Automated) Leitner *et al*[42]	Type of advanced cell separator designed to produce fibrin sealant It is cumbersome, expensive, have low and damaged platelet yielding capacity
	Anitua's PRGF# (Manual) Anitua[43]	Citrated blood is collected in 5 mL tubes and softly centrifuged for 8 min at 460 g Platelet poor layer (1 mL) is discarded and the PRGF layer above buffy coat layer is pipetted out from all tubes and collected in one tube. Calcium chloride is added for clotting. However there are problem in ergonomy and reproducibility of the procedure
	Nahita PRP (Manual) Tamimi *et al*[44]	Protocol similar to Anitua's PRGF
L-PRP	PCCS PRP (Automated) Weibrich *et al*[45]	Consists of two compartments, citrated blood is transferred into first compartment and centrifuged for a short time. Using air pressure, upper layer PPP and buffy coat are transferred into second compartment and centrifuged for a longer time. PPP is transferred back to first compartment and final product - leukocyte and PRP is left behind. It is no longer available
	SmartPReP PRP (Automated) Weibrich *et al*[46]	It also has two compartments, but requires less manipulation It is a multifunctional system which can also be used to concentrate stem cells from bone marrow transplant
	Megalian APS PRP (Automated) Christensen *et al*[47]	This advanced cell separator had optical reader. It has compact size, designed for small blood samples (upto 50 mL). Although, platelet collection efficacy is high but cell preservation is yet to be known
	GPS PRP (Automated) Martovits *et al*[48]	Another variation of 2 chambers, 2 stage centrifuge protocol PPP is discarded and second centrifuge is with RBC layer. Final PRP is aspirated from the surface of RBC layer
	Friadent PRP (Manual) Weibrich *et al*[46]	Both these techniques employ classical method of 2 stage centrifuge. First soft spin that gives three layers. PPP and buffy coat transferred to another tube and after hard spin the PPP is discarded leaving behind PRP
	Curasan PRP (Manual) Weibrich *et al*[50]	Depending on technique of collecting buffy coat, one can randomly get either P-PRP or L-PRP
	AutoloGel (Automatic) Driver *et al*[49]	The final product was called as "autologous platelet-rich plasma gel"
	Regen PRP (Manual) Plateltex PRP (Manual) Mazzucco *et al*[41]	Both these techniques uses specific jellifying agents such as calcium gluconate and lyophilized purified batroxobin, an enzyme that cleaves fibrino-peptide to induce fibrin polymerization without bovine thrombin and gelling in about 10 min[47] The Regen method also employs a separator gel within the centrifugation tubes to improve collection of platelets and leucocytes
	Ace PRP (Manual) Tamimi *et al*[44]	Similar protocol but with variation in centrifugation force and time and types of anticoagulant
P-PRF	Fibrinet PRFM (Manual) (PRFM Kit, Cascade Medical, New Jersy, United States) Leitner *et al*[42]	Consists of two tubes, one for blood collection and another for PRFM clotting. Around 9 mL blood is collected in a tube containing tri-sodium citrate anticoagulant and a separator gel and centrifuged for 6 min at high speed. Buffy coat and PPP are transferred in second tube containing calcium chloride and centrifuged for 15 min and then stable PRFM clot can be collected. Very low amount of leucocytes are obtained due to the specific separator gel used, however the fibrin matrix is more denser and stable than PRP's
L-PRF	Choukroun's PRF (Manual) Choukroun *et al*[22]	Considered second generation platelet concentrate obtained by natural process without any anticoagulants or jellifying agents Venous blood collected and centrifuged at low speed yielding and RBC layer, PRF clot in middle and acellular plasma top layer The PRF clot can be pressed between guage to make a strong membrane
	Intra-Spin[9] (Manual) (Intra-lock, United States)	The only FDA approved kit for PRF. It employs 9 mL glass coated plastic tube, centrifuged at room temperature at 2700 rpm (around 400 g) for 12 min. Contains and Xpression kit to compress the clot to make membranes
	Titanium-prepared PRF (experimental) (Manual) Tunalı *et al*[33]	The platelet activation by using titanium tubes instead of glass tubes seems to offer some high characteristics to T-PRF The PRF obtained was highly organized and with continuous integrity. The fibrin meshwork is thicker and covers larger area
	Other non FDA cleared centrifuge to produce L-PRF: Salvin 1310 (Salvin Dental) and LW-UPD8 (LW Scientific)	Studies have shown that as compared to Intra-spin, these 2 machines produces more vibration and resonance

CGF	Medifuge, Silfradent srl, Italy Sacco[26]	Permits the isolation of a much larger, denser fibrin matrix which is richer in growth factors Demonstrates presence of TGF-b, VEGF and $CD34^+$
Sticky Bone	Sohn[29]	Autologous fibrin glue mixed with bone graft
T-PRF	Tunalı *et al*[33]	Titanium tubes were used for collection and centrifugation instead of glass tubes
A-PRF	(Advanced PRF Process, France) Choukroun[32]	Earlier vascularization, faster soft tissue growth, more cytokines and release of BMPs
i-PRF	Mourao *et al*[34]	Blood collected in 9 mL tube without any additive, centrifuged for 2 min at 3300 rpm, the resultant orange color fluid in the tube is the i-PRF

PCCS: Platelet concentrate collection system; APS: Autologous platelet separator; PRP: Platelet-rich plasma; PRGF: Plasma rich in growth factors; PRF: Platelet-rich fibrin; TGF: Transforming growth factor; VEGF: Vascular endothelial growth factor; GPS: Gravitational platelet separation system.

positive effects on gingival fibroblasts[56], oral osteoblasts[57], and periodontal ligament (PDL) fibroblasts[58], making it an ideal candidate to facilitate complete periodontal regeneration. PRP may also benefit surgical sites and wound healing *via* its antibacterial properties. This antimicrobial effect has been reported against bacteria such as *Staphylococcus aureus*[59], *Escherichia coli*[60], and *Klebsiella pneumonia*[61]. PRP was also found to be active against oral microorganisms, including *Enterococcus faecalis, Candida albicans, Streptococcus agalactiae,* and *Streptococcus oralis*[62], reinstating that PRP is a potentially useful substance in fighting postoperative infections.

Applications in periodontics

Application of PRP to bone graft material has demonstrated earlier bone regeneration and soft tissue healing[21]. PRP can also retard epithelial migration by infusing it into resorbable barrier membranes. This will also provide localized source of growth factors that will accelerate soft tissue and hard tissue maturation[63]. Agrawal and Gupta[64] (2014) in a split mouth study concluded that a combination of PRP with DFDBA was more efficient than DFDBA with saline for the management of non-contained intrabony defects. In addition to this, a combination of PRP with bovine porous bone mineral and GTR membrane also showed good clinical response[65]. Combination of PRF and bone graft has also reported exceptional results in periodontic-endodontic furcation defect[66]. However, Choi *et al*[67] questioned the benefits of mixing PRP and bone graft material, expressing their concern that it interfere new bone formation. According to the authors, growth factors when present in high concentrations at inappropriate times for prolonged duration can negatively affect the cell behavior. They further affirmed that proliferation and viability of alveolar bone cells are quashed by high PRP concentrations but are accelerated by low PRP concentrations[68].

PRF is a powerful healing biomaterial with inherent regenerative capacity and can be used in various procedures such as periodontal intrabony defects[69,70], treatment of furcation[71], sinus lift procedures[72] and as application in the field of tissue engineering, it can be used as a scaffold for human periosteal cells *in vitro*[73]. Eren and Atilla[74] in 2012 treated bilateral gingival recession with (CAF) coronally advanced flap and (SCTG) subepithelial connective tissue graft on one side and CAF with PRF on other side. They found improvement in all parameters with both the techniques. Since use of PRF was practical and simple to perform and also eliminates the requirement of donor site wound, they suggested that CAF + PRF as a better alternative to CAF + SCTG. Anilkumar *et al*[75], reported PRF as a probable but innovative approach for root coverage in treating gingival recession in mandibular anterior region using combination of PRF membrane and laterally positioned flap technique. Aroca *et al*[76] in a randomized clinical trial concluded that addition of a PRF membrane placed under the MCAF (modified coronally advanced flap) provided additional gain in gingival/mucosal thickness but inferior root coverage over 6 mo follow up period compared to the conventional therapy.

Applications in implantology

Choi *et al*[77] in 2006 conducted an animal study to compare the sinus lining perforation repair using either the (AFG) autologous fibrin glue or the collagen membrane. Their histological evaluation found that in repaired wounds, where AFG was used, demonstrated newly regenerated continuous epithelium across the original perforation site as compared to collagen membrane treated site where there was no epithelium, inflammatory infiltration was seen along with extensive fibrosis even after 2-wk of healing. Literature reports the applications of PRP in continuity defects[78], sinus lift augmentation[79,80], vertical/horizontal ridge augmentations[81], ridge preservation[82], periodontal/peri-implant defects[83]. Several articles have reported the use of L-PRF membranes for the stimulation of bone and gingival healing during sub-antral sinus augmentations[72] and global rehabilitations using dental implants[84,85]. The effect of these membranes on soft tissue healing and maturation is particularly significant[86]. In yet another case report, Del Corso *et al*[87] in 2012 used L-PRF in immediate implant replacement of maxillary central incisor and reported excellent healing and esthetics. Choukroun *et al*[88] studied the effect of PRF with (FDBA) freeze-dried bone allograft to augment bone regeneration in direct sinus lifting and found accelerated bone regeneration.

Simonpieri *et al*[84,85], in a two-part publication, reported an innovative technique for maxillary reconstruction using PRF membranes, FDBA and 0.5% metronidazole solution. A 0.5% metronidazole solution (10 mg) in small quantity provides an effective shielding of the bone graft material against unavoidable bacterial contamination[89]. The membrane component of PRF was used to guard the surgical site and enhance the soft tissue healing.

However the PRF fragments were blended with the graft particles. They also suggested that the PRF membranes can be trimmed into fragments (millimeter size) and added to graft material, functioning as a "biological connector" between the different elements of the graft, and will form a matrix which will promote the migration of osteoprogenitor cells to the center of the graft, neo-angiogenesis and capture of stem cells[90,91]. Using the protocol reported in the literature, they frequently observed a greater degree of gingival maturation post-healing. They also noticed thickening of keratinized gingival tissues that eventually enhanced the esthetic integration and final result of their prosthesis. Moreover, all their clinical experiences highlighted that the use of PRF seemed to reduce postoperative edema and pain, and even minor chances of infectious phenomena[85]. PRF can be condensed to make plugs which can be positioned in the implant osteotomy site to promote sinus floor elevation using a crestal core elevation (CCE) procedure[92] or osteotome-mediated sinus floor elevation (OMSFE) with simultaneous implant placement[93]. PRF can not only be used as a substitute for particulate grafting to predictably elevate the sinus floor using a crestal approach, but PRF can also provide protection for the sinus membrane during the use of an osteotome. Even in case of sinus membrane perforation, the fibrin matrix can aid in wound closure[77,94]. PRF plugs can also be indicated in management of residual extraction sockets[95]. A technique in which autologous PRF is used in extracted socket after immediate bone augmentation using titanium membranes applied to the socket walls and achieving primary closure, was found to be feasible and safe with adequate bone filling after 8 wk or above for implant fixation[96]. Hafez *et al*[97] in 2015 demonstrated that PRF membrane maintains particulate autogenous bone graft and help achieve primary coverage over immediately placed implants. Sohn *et al*[53] compared CGF membrane and collagen membrane for alveolar ridge augmentation. Their bone biopsy results showed favorable new bone formation along mineral allograft without sign of inflammation. They also evaluated three dimensional ridge augmentation using sticky bone with or without use of titanium mesh, and found favorable augmentation even without the use of titanium mesh[53].

The use of platelet and immune concentrate during bone grafting offers the following 4 advantages[85]: Firstly, the fibrin clot plays an important mechanical role, wherein the PRF membrane maintains and protects the bone graft and its fragments, when incorporated in the body of bone graft, serving as biological connectors between bone particles. Secondly, the fibrin network promotes cellular migration, particularly for endothelial cells which are necessary for the neo-angiogenesis[40], vascularization and survival of the graft. Thirdly, the platelet cytokines (PDGF, TGF-beta, IGF-1) are creating a perpetual process of healing gradually released as the fibrin matrix is resorbed[84,98]. Lastly, the leukocytes and cytokines in the fibrin network play a significant role in the self-regulation of inflammatory and infectious phenomena within the grafted material[99].

DISCUSSION

In preparation of PRP, the choice of anticoagulant used is an important parameter in its capability of preserving the platelets' best possible functionality, integrity and morphology. In particular do Amaral *et al*[100] (2016) concluded that the use of (EDTA) ethylene-diamine-tetra-acetic acid yielded more platelet in whole blood; however, it increased the mean platelet volume (MPV) following the blood centrifugation steps required for obtaining PRP. Authors also discovered that the use of (ACD) anticoagulant citrate dextrose and sodium citrate (SC) significantly induced mesenchymal cell (MSC) proliferation. Moreover, PRP obtained in sodium citrate anticoagulant not only presented higher platelet recovery after the first centrifugation step but also had a minimal change in MSC gene expression. Citrate seems to be a suitable anticoagulant, because it has been recently shown that thrombin-activated PRP releases all growth factor at the same time in a bolus, while non-activated PRP uses the platelets as a sustained delivery system, exhibiting the best wound healing effects[101]. PRP is not routinely used nowadays because of complicated preparation techniques, expensive procedure and offer quite mixed clinical results[2,3]. On the other hand, the L-PRF family has developed very fast over the last years, as the technique is very simple and useful in daily practice, it is user friendly and relatively inexpensive[11].

One logical question that comes to a clinician is how much rich is PRP or PRF? What is the difference of richness in these PC's? Literature reports a range of less than 2 fold to around 8.5 fold increase in platelets. In a classification of PRP, Mishra *et al*[30] suggested a sub-classification of PRP into A and B, where a 5-fold platelet concentrate may be a relevant baseline for definition of PRP (it should also be noted that concentrations greater than 5-fold gave better clinical results). Another aspect of this definition is that this baseline is not universal and may not be valid for all clinical applications. Weibrich *et al*[102] suggested that different individuals may require different platelet concentration ratios to achieve comparable biological effect.

Although leukocyte rich and leukocyte poor PRP's have their own place in literature, the importance of non-platelet components in a platelet concentrate remains a mystery. Parrish *et al*[103] 2016, in an *in-vitro* study demonstrated that leukocyte poor PRP (LP-PRP) showed poor coagulation and poor platelet growth factor release compared to whole blood and leukocyte rich PRP (LR-PRP). They also checked tendon cell proliferation *in-vitro* using serum from LP-PRP and LR-PRP and found greater advantages with the later. LP-PRP was inferior even to whole blood. Thus they concluded that cellular components other than platelet, that are usually eliminated during the course of PRP preparation, are important for efficient functioning of platelets including its thrombin generation, growth factor release and capacity for cell proliferation[103]. However, these findings need to be confirmed *in-vivo* to make them more justifiable. In addition to this, difference in the

age of patient from who's blood PRF is made also differs structurally and qualitatively. In a recent study, Yajamanya *et al*[104] (2016) evaluated fibrin network pattern changes of PRF in young and old age groups using a cell-block cytology method. They found that in progressing age groups there was significant decrease in dense and increase in loose fibrin network. They also discovered reduction in the number of platelets and WBC's entrapped within fibrin network with increasing age groups.

It has always been a common thought that L-PRP or L-PRF would give an additional advantage over P-PRP or P-PRF due to the presence of immune cells, *i.e.*, leukocytes. Does that mean that platelets do not have any role to play in immunity? Numerous studies have emphasized that human platelets are a good source of antimicrobial peptides such as: Thymosin β4, platelet basic protein, platelet factor 4, connective tissue activating peptide Ⅲ, fibrino-peptides A and B and chemokine (C-C motif) ligand 5[105]. There are special receptors on the platelets that are known to aggregate with bacteria. Platelets also participate in generating oxygen metabolites, including hydrogen peroxide, superoxide, and hydroxyl free radicals[106]. Largely, platelets demonstrate impressive activities against the blood-borne pathogens and also play an important role in the innate host defense against the initiation and progression of infections[106]. In fact Garraud *et al*[107] in 2015 claimed that "platelets are innate and inflammatory cells and do not only assist immunity but are immune cells themselves". Anitua *et al*[61] demonstrated that even if an additional dose of leukocytes was present it did not significantly enhance the antimicrobial properties of PRP. Yang *et al*[108] (2015) in a study evaluated the antimicrobial activity of four plasma preperations: PRP, platelet poor plasma (PPP), platelet depleted plasma (PDP) and PRF. Using haemocytometer, they found leucocytes only in PRP and not in other preparations. However, their results showed that all plasma preparations were efficient enough to inhibit bacterial growth for > 24 h with PRP as the strongest antimicrobial agent. In terms of time-kill assay, authors discovered that PRP, PPP and PDP had similar effect on *F. nucleatum* indicating that it was sensitive to the antibacterial agents in plasma. The poor antimicrobial effect of PRF was attributed to the fact that a mesh of fibrin was formed in PRF, which adsorbed these agents and thus exerted less minimal effect on the growth inhibition of this microorganism. However, one should note that the technique of PRF preparation was not according to the L-PRF protocol given by Choukroun *et al*[22] in 2000. To make PRF, Yang *et al*[108] used fraction of PRP and activated it by 23 mmol/L of calcium chloride for 30 min and centrifuged again at 6000 g for 30 min to recover "fibrin-free supernatant" which they labeled as PRF. Hence, although their experiment highlighted the antimicrobial effect of plasma, regardless of platelet and leukocyte concentration, their conclusion of PRF should be read with caution. The basic biological difference between PRP and PRF is that in PRP the polymerization is artificially provoked and there is extrinsic growth factor enmeshment, whereas in PRF there is natural polymerization with intrinsic growth factors enmeshment. When compared *in-vitro*[109] studies have revealed that most of the growth factors from P-PRP gel are released in the first hours after preparation and get completely dissolved in the medium after 3-d. In contrast the L-PRF membrane not only remained intact and solid after 7-d but also continuously released large quantities of growth factors. These growth factors are sustainably released for at least 1 wk up to 28 d[110]. This allows PRF to stimulate the environment for a significant time during wound healing. As a general concern, at the time of any surgery, platelets will start collecting at the surgical site to initiate clotting and healing, which may reduce the whole blood platelet count[111]. As such, it is recommended that blood should be drawn before the surgery starts because the surgery itself might cause platelet activation and that may eventually interfere with preparation of platelet concentrate[112,113]. Also the massive release of TSP-1 from PRF membrane has opened up a new range of application for this membrane[8].

Considering technical aspects for preparation of PRP, for the first centrifuge it is best to keep the speed and time to the shortest that will separate the RBC's and plasma clearly. In the second centrifuge the time and speed should be sufficiently high so that more platelets will precipitate without destroying them[39]. Ehrenfest *et al*[114], claimed that for small table centrifuges, the most relevant parameters to be logically evaluated was the vibrations of those centrifuge, the vibration shocks at the time of acceleration and the eventual resonance. All these mechanical properties may impede with the quality and biological signature of the final L-PRF product. The authors tested 4 different centrifuges; *viz*: The original L-PRF centrifuge (Intra-Spin, Intra-Lock) and 3 other laboratory centrifuges: Salvin 1310 (Salvin Dental), LW - UPD8 (LW Scientific) and the A-PRF 12 (Advanced PRF, Process). They demonstrated even if the centrifuges were used in the same conditions and at the same speed there was a significant discrepancy in their vibration levels and 3 out of four quickly reached a threshold of resonance. They found "Intra-Spin" to be the most stable machine tested. At the traditional speed of production of L-PRF, the level of undesirable vibration was between 4.5 and 6 times lower with this machine than with other centrifuges. Moreover, Intra-Spin always stayed under the threshold of resonance, as compared to the other three tested machines[114].

CONCLUSION

There have already been many technological advancement in preparing and understanding the various types of PC from random single spin centrifugation to fully automated commercially available systems. However, the characterization of such complex products seems to remain incomplete due to the number of parameters involved. Apart from presence or absence of leukocytes, whether or not the activation is carried out, other

parameters that should be taken into consideration are the quantity or rate of platelet collection, the quantity and rate of leukocyte collection, cell composition and preservation during collection, transportation and centrifugation. As discussed earlier, the parameters particular to the centrifuge used are also important such as: Its size, vibration, the duration of centrifugation. Other than that, the cost involved, ergonomics, the form and volume of final product, *etc*., also need to be taken into consideration while evaluating newer techniques, commercial products, classification systems or indications for their application in medicine and dentistry. With L-PRF being more user friendly and economic, this arsenal is finding wider applications in surgical field. The introduction of i-PRF will also find suitable applications, where injectable form of platelet concentrate is required. Looking at the current trends PRP and L-PRF are most commonly used and have been researched upon. Newer advances such as A-PRF, i-PRF, t-PRF, CGF and sticky bone concept have been reported in single or few cases but no long term or controlled trial have been done to prove the advantage of their advancement over conventional PRP and PRF. So clinicians should use the advancements with caution.

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P- Reviewer: Rattan V, Tatullo M **S- Editor**: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Clinical Cases

Submit a Manuscript: http://www.f6publishing.com

World J Clin Cases 2017 May 16; 5(5): 172-177

DOI: 10.12998/wjcc.v5.i5.172

ISSN 2307-8960 (online)

ORIGINAL ARTICLE

Observational Study

Robotic single-site supracervical hysterectomy with manual morcellation: Preliminary experience

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Author contributions: Ding DC design the study, acquisition and interpretation of the data, draft manuscript; Hong MK, Chu TY, Chang YH and Liu HW revised the article critically for important intellectual content.

Institutional review board statement: This study was reviewed and approved by the Research Ethics Committee of Buddhist Tzu Chi General Hospital.

Informed consent statement: This study is a retrospective chart review and approved by IRB. Therefore, no informed consent was needed.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data available.

Manuscript source: Invited manuscript

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Received: September 30, 2016

Peer-review started: October 10, 2016

First decision: December 1, 2016

Revised: December 8, 2016

Accepted: February 28, 2017

Article in press: March 2, 2017

Published online: May 16, 2017

Abstract

AIM

To evaluate the feasibility, safety and peri- and post-operative outcomes of robotic single-site supracervical hysterectomy (RSSSH) for benign gynecologic disease.

METHODS

We report 3 patients who received RSSSH for adenomyosis of the uterus from November 2015 to April 2016. We evaluated the feasibility, safety and outcomes among these patients.

RESULTS

The mean surgical time was 244 min and the estimated blood loss was 216 mL, with no blood transfusion necessitated. The docking time was shortened gradually from 30 to 10 min. We spent 148 min on console operation. Manual morcellation time was also short, ranging from 5 to 10 min. The mean hospital stay was 5 d. Lower VAS pain score was also noted. There is no complication during or after surgery.

CONCLUSION

RSSSH is feasible and safe, incurs less postoperative pain and gives good cosmetic appearance. The technique of in-bag, manual morcellation can avoid tumor dissemination.

Key words: Robotic surgery; Single-site; Supracervical

hysterectomy; Single port; Subtotal hysterectomy

Core tip: Robotic single-site surgery (RSS) is feasible and safe in performing supracervical hysterectomy for benign gynecologic disease. Less pain and cosmetic value are important advantages of RSS. Manual morcellation can be done through single port setting.

Ding DC, Hong MK, Chu TY, Chang YH, Liu HW. Robotic single-site supracervical hysterectomy with manual morcellation: Preliminary experience. *World J Clin Cases* 2017; 5(5): 172-177 Available from: URL: http://www.wjgnet.com/2307-8960/full/v5/i5/172.htm DOI: http://dx.doi.org/10.12998/wjcc.v5.i5.172

INTRODUCTION

The first laparoscopic subtotal hysterectomy (LSH) was reported in 1991[1]. Retaining the cervix may preserve sexual, urinary and bowel function[2].

LSH is approached in the same manner as total laparoscopic hysterectomy (LTH). After uterine vessels are secured, the cervix is transected at the level of internal os. However, the ascending branch of uterine vessel is sometimes hard to approach. During transection, severe bleeding may occur. Amputation of the cervix is also a time-consuming procedure. The loop is also designed for cervical amputation and could save 80% of the time required for performing this procedure[3]. Retrieval of uterine corpus after the transection was achieved by mechanical or manual morcellation through an extended abdominal port[4]. The mean surgical time of LSH ranged from 70 min to 134 min[5]. Complications and outcomes are comparable with those of LTH. Above all, the technique involved in LSH is more difficult than LTH because of the time required for amputation of cervix.

Robotic assisted hysterectomy (RAH) has been increased from 0.5% in 2007 to 9.5% in 2010[6,7]. Although RAH is a safe approach to hysterectomy, but the longer surgical time required[8-10]. Compared to open surgery, RAH provides advantages for reduced length of hospital stay and blood transfusions[11].

Laparo-endoscopic single-site surgery (LESS) offered a new way to perform minimally invasive gynecological surgery[12-14]. The advantages of LESS included less postoperative pain, lower dosage of analgesic required[13], greater cosmetic satisfaction[14], lower morbidity and comparable outcomes compared with those of standard laparoscopic surgery[14,15]. Nevertheless, LESS involves technical challenges such as loss of port triangulation, clashing of instruments and long learning curve. Robotic single-site surgery (RSS) may provide advantages to overcome these shortages[16,17].

Table 1 Characteristics of patients received robot single-site supracervical hysterectomy

Patient	1	2	3	Mean
Diagnosis	Adenomyosis	Adenomyosis	Adenomyosis	
Age (yr)	44	43	48	45
BMI (kg/m^2)	22.5	23.6	26.6	24.2
Previous surgery	Partial oophorecotmy	Nil	C/S	
Largest diameter of uterus (cm)	8	10	11.9	10
Total op time (min)	200	233	300	244.3
Docking time (min)	30	20	10	20
Console time (min)	120	160	165	148.3
Morcellated time (min)	5	5	10	6.7
Blood loss (mL)	100	300	250	216.7
VAS (1 h)	3	4	4	3.7
VAS (24 h)	3	4	2	3
VAS (48 h)	0	2	0	0.7
Hospital stay (d)	4	4	4	4
Complication	0	0	0	0

VAS: Pain score; BMI: Body mass index.

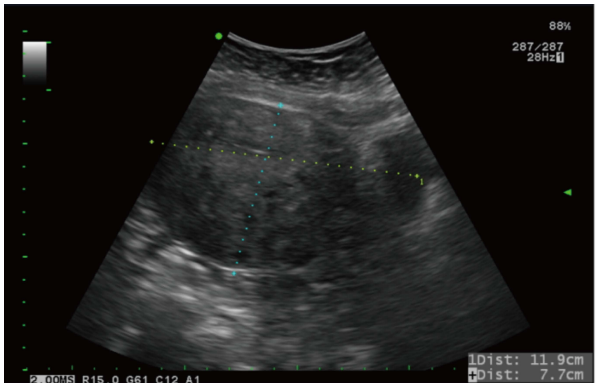

Figure 1 Ultrasound of adenomyosis of uterus. The largest diameter of uterus measured was 11.9 cm.

Here we described supracervical hysterectomy performed with single-site da Vinci Surgical System (Si version, Intuitive Surgical, Sunnyvale, CA, United States) in three patients affected by adenomyosis of the uterus.

MATERIALS AND METHODS

Three women presented with adenomyosis of the uterus complicated with menorrhagia and dysmenorrhea. Two patients had previous history of abdominal surgery. One woman had anemia (Hb: 10.3 g/dL) (Table 1).

Abdominal ultrasound was performed for all patients; their maximum diameters of uterus were listed in Table 1. Figure 1 shows the uterus of the largest diameter of 11.9

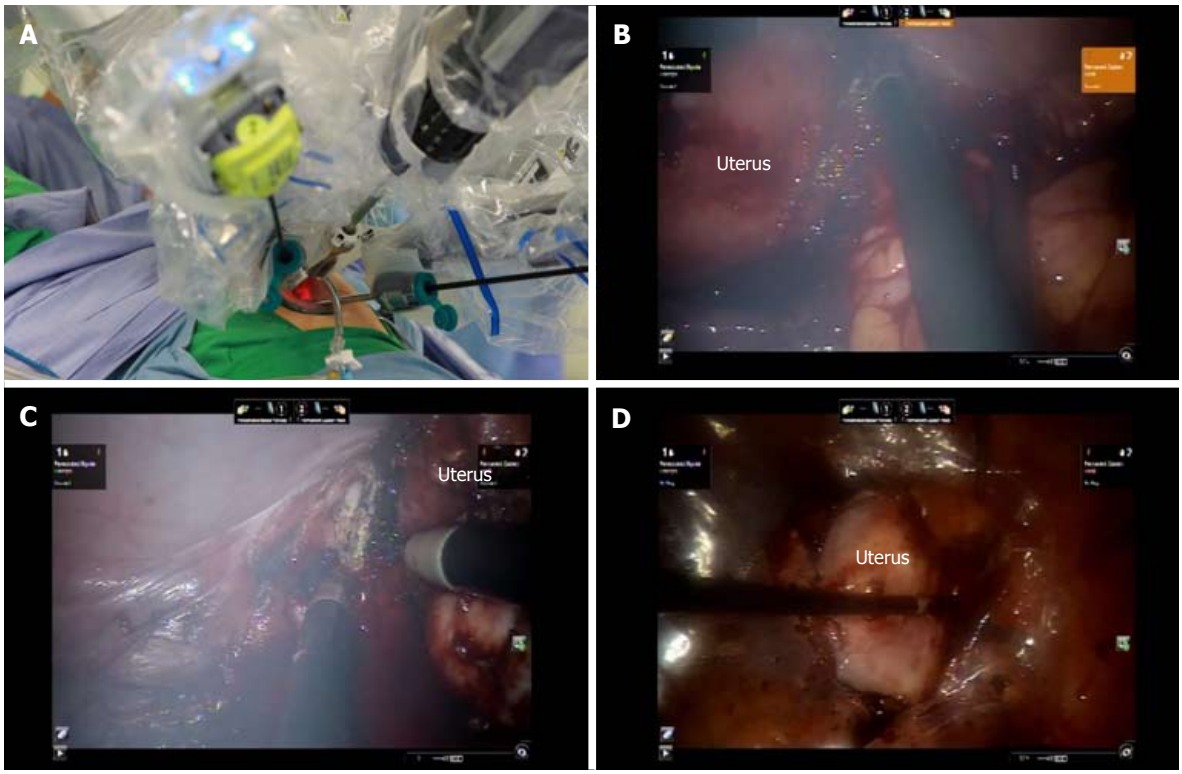

Figure 2 Intraoperative view of supracervical hysterectomy. A: Placement of robotic trocars using a single-site device; B: Cutting right cervical region; C: Cutting left cervical region; D: Amputated uterus placed into tissue bag.

cm with the suspected lesion of adenomyosis located at the posterior uterine wall.

The patients were then scheduled for robotic-assisted supracervical hysterectomy. The single-port device is a multichannel non-reusable specific port with space for four cannulas and an insufflation valve. A target anatomy arrow indicator is marked on the cannula. Two 25-cm curved cannulas for robotic instruments, one cannula for the high-definition three-dimensional endoscope, and one 5-mm assistant cannula were used in the surgery.

The uterine manipulator was placed to adjust the uterine position. After catching the bilateral skin along the umbilicus with two Allis clamps, a 2-cm midline umbilical skin incision was made. Through this incision, a wound retractor (Lagis, Taichung, Taiwan) was introduced into the abdominal cavity, then a single-site port (da Vinci Surgical System) was introduced into the abdominal cavity grasped by an atraumatic clamp through the wound retractor.

The patient was placed supine in lithotomy position with 30° Trendelenburg position, and the robotic patient cart was positioned between the patient's legs. Then the robotic arms were opened in the opposite position. The 30° endoscope was placed in camera trocar and a watchful inspection of total abdominal cavity was performed.

Then the other three cannulas were inserted through the port and their positions were adjusted according to the scope view and mark. The remaining cannula was placed in front of the uterus and then held still to allow docking. Finally, robotic instruments including fenestrated bipolar and hook unipolar instruments were introduced (Figure 2A). One Veress needle (COVIDIEN) was inserted at suprapubic region under direct vision by endoscope for evacuation the smoke. After cutting both right and left endocervical regions (Figure 2B and C), the amputated uterus was rolled and placed into a tissue bag (Cook, Figure 2D). Then the robot was undocked and the tissue bag was grasped to the umbilical port using an assistant port grasper. Then the uterus was manually morcellated from the umbilical wound (Figure 3A) and all morcellated pieces were placed onto a plate (Figure 3B). Then one sheet of Seprafilm was cut into four pieces and placed with or without docking robot arms onto surgical sites to prevent adhesion (Figure 3C). After all robotic procedures were completed, the umbilical wound was closed using interrupted 0 Vicryl for the fascia layer and 3-0 Vicryl for the subcutaneous layer (Ethicon, Figure 3D).

Statistical analysis

Statistics using Student's *t*-test was performed when compare pain score of the two groups, and the differences between the groups were considered significant at $P < 0.05$.

RESULTS

The mean operative time was 244 min and the estimated blood loss was 216 mL (Table 1), with no blood transfusion necessitated. The docking time was shortened gradually

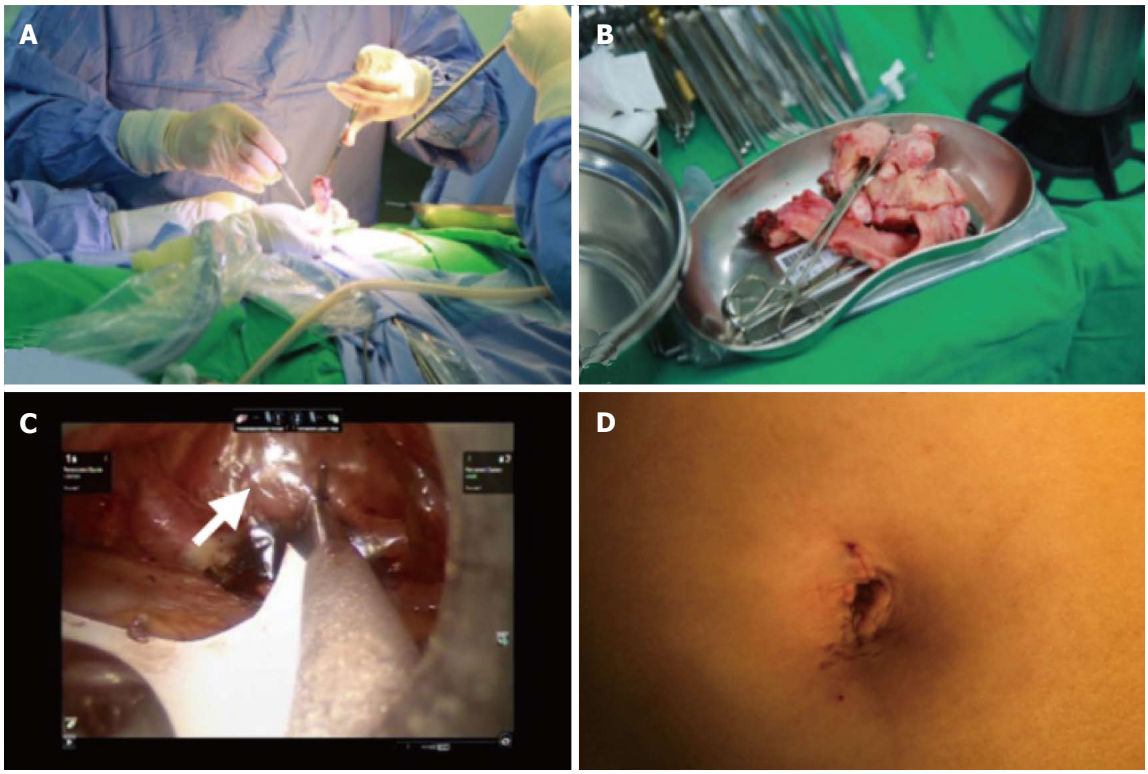

Figure 3 Intraoperative view of manual morcellation of the uterus and the placement of seprafilm. A: Manual morcellation of uterus through the single-site wound; B: Morcellated uterus; C: Seprafilm placed onto surgical sites (arrow); D: Postoperative umbilical scar.

from 30 to 10 min. We spent 148 min on console operation. Manual morcellation time was also short, ranging from 5 to 10 min. The post-operative course was uneventful and all patients were discharged 3 d after operation. The VAS pain score was 3.7, 3.0 and 0.7 at 1, 24 and 48 h, respectively. The mean hospital stay was 4 d. The surgical specimens conformed adenomyosis of the uterus. There is no complication during or after surgery.

DISCUSSION

Single-site surgery has become popular due to improved cosmetic appearance, multiple incisions avoided, and minimal post-operative pain and recovery time[13,14]. Nevertheless, LESS surgery is characterized by longer surgical time and technical challenge. Robotic single-site surgery (RSS) is the same as LESS, but the instrument was more ergonomic compared with other single-site methods[18,19]. In our experience, RSS supracervical hysterectomy (SH) is a valid alternative to laparoscopic and standard robotic SH and provides the same surgical outcome.

There is only one study report on the RSSSH experience in gynecology[20]. However, there is no detailed information regarding RSSSH except the number of patients while there are several reports on RSS hysterectomy (RSSH)[16,18,21-23]. RSSH was first reported in 2011[23] and concluded to be feasible offering several advantages such as smaller scar, less pain and the same outcome compared with standard robotic surgery[23]. Moreover, in preclinical models of human cadavers, the RSS technique is effective and reproducible in various gynecological surgeries[24].

There is a more surgical time in RSSSH than in RSSH. The total surgical time is 134 min in RSSH but 244 min in RSSSH[19]. The cause of more surgical time may be attributed to our initial experience and the type of surgery performed. The pelvic adhesiolysis have also contributed to longer operating time. A lot of surgical time was spent in the endocervical ring cutting. The cutting efficiency of robot hook is not efficient. Coagulate the bleeding caused by cutting the endocervical ring is also time consuming. However, we assume the surgical time can be shortened after more surgical experiences.

There is more blood loss after RSSSH than after RSSH[19]. The mean blood loss is 50 mL in RSSH but 240 mL in RSSSH. The cause of greater blood loss may be attributed to our initial experience and the type of surgery performed. In RSSH, the vagina is cut after securing the uterine vessels. However, in RSSSH, the ascending branch of uterine vessels cannot be easily secured using a bipolar instrument. Therefore, after cutting the bilateral endocervical region, bleeding can sometimes be vigorous. This condition is the same for LESS supracervical hysterectomy[25].

The advantage of RSS is less post-operative pain, thus necessitating less pain control[13,14]. This study also demonstrated these advantages. The VAS pain score was 3.7, 3.0 and 0.7 at 1, 24 and 48 h, respectively. In contrast, the VAS in LESS hysterectomy was 5.6, 3.7 and

Table 2 Comparison of postoperative pain

Time	LESS hysterectomy (n = 36)	RSSSH (n = 3)	P value
VAS pain score			
0-2 h	5.68 ± 2.11	3.7 ± 0.6	< 0.05
24 h	3.75 ± 1.61	3.0 ± 1.0	> 0.05
48 h	2.25 ± 1.59	0.7 ± 1.6	< 0.05

LESS: Laparoendoscopic single-site surgery; RSSSH: Robot single-site supracervical hysterectomy; VAS: Visual analog scale.

2.2 at 1, 24 and 48 h, respectively (Table 2)[13], indicating significantly lower VAS pain score in RSSSH than in LESS hysterectomy at 1 and 48 h (P < 0.05). Infiltration wound with ropivacaine or other long-acting local anesthetics also provide good pain control[19,26].

The mean hospital stay in this study is 4 d. Nevertheless, the hospital stay is only 3 d in the previous study[19]. The long hospital stay in our study is due to the health insurance in our country. The insurance offers the patient can stay in hospital for 4 d.

Power morcellation had been widely used in laparoscopic surgery to speed removal of specimen[27]. However, owing to the risk of leiomyosarcoma dissemination after power morcellation, removal of specimen in a bag was suggested[28,29]. Therefore, techniques for safe specimen removal have been reported[30]. We also developed a technique of manual morcellation[31]. In this study, we used the same technique for placing the specimen into a tissue bag and for manual morcellation through the single-port wound. This morcellation method is relatively safe without tumor cell or tissue dissemination.

The use of Seprafilm as adhesion barrier was approved by the FDA in 1996. However, Seprafilm is seldom used in laparoscopic surgery because it easily breaks and sticks[32]. We applied a simple technique (using wet gauze and paper roll) for rapid and safe placement of Seprafilm onto the surgical sites[33].

Another problem encountered during RSS is surgical smoke that could influence the vision. With RSS using both unipolar and bipolar energies, there is no additional port for passage of smoke in the single-port device. To overcome this problem, a small Veress needle is used for smoke release, thus achieving good vision outcome.

In conclusion, we demonstrated that RSSSH is feasible and safe in gynecologic patients. Less postoperative pain and greater cosmetic satisfaction were the major advantages of RSSSH. The technique of in-bag, manual morcellation could avoid tumor dissemination. Nevertheless, randomized study and the outcome of long-term follow-up are still needed in the future.

COMMENTS

Background

Minimally invasive surgery has been popular in gynecologic surgery. Therefore, despite conventional multi-port laparoscopic surgery, laparoscopic single-site surgery (LESS) emerges since 2009. However, there are some technical difficulties and instrument design hurdling the progress of LESS. Nevertheless, Robotic single-site surgery (RSSS) solves the technical and instrument problems in LESS.

Research frontiers

RSSS is in its beginning stage. Although there are several papers discussing the RSSS, there is still a lot of space to improve the RSSS on supracervical hysterectomy (SH). The authors attempted to use RSSS to perform SH and to test if RSSS is a feasible and safe method to perform SH.

Innovations and breakthroughs

The present study demonstrated RSSSH is a feasible and safe method for the patients with adenomyosis of the uterus.

Applications

The data in this study suggested that RSSSH could be a feasible and safe modality for patients with adenomyosis of the uterus.

Terminology

Adenomyosis of the uterus is a condition of endometrial glands presented in the myometrium and enlarged of the uterus. The symptoms of adenomyosis are including dysmenorrhea and menorrhagia that cause the major reason for women receiving hysterectomy.

Peer-review

The authors investigated the feasibility of RSSSH for adenomyosis of the uterus and found that this approach is safe and acceptable in the management of the similar patients in the future based on the analysis of outcome from the 3 patients.

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P- Reviewer: Blumenfeld Z, Cosmi E, Rovas L, Wang PH
S- Editor: Gong ZM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Clinical Cases

Submit a Manuscript: http://www.f6publishing.com

World J Clin Cases 2017 May 16; 5(5): 178-182

DOI: 10.12998/wjcc.v5.i5.178

ISSN 2307-8960 (online)

CASE REPORT

Ticagrelor therapy and atrioventricular block: Do we need to worry?

Elia De Maria, Ambra Borghi, Letizia Modonesi, Stefano Cappelli

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Author contributions: De Maria E contributed to conception and design of the work, drafting the article, final approval; Borghi A, Modonesi L and Cappelli S contributed to drafting and critical revision of the work, final approval.

Institutional review board statement: This case report/editorial was exempt from the Institutional Review Board standards at our Institution.

Informed consent statement: The patients involved in this study gave their oral informed consent authorizing use and disclosure of their protected health information. At our Institution informed oral consent is regarded as sufficient for case reports/editorial.

Conflict-of-interest statement: The authors report no relationships that could be construed as a conflict of interest.

Manuscript source: Invited manuscript

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Received: January 8, 2017
Peer-review started: January 12, 2017
First decision: February 17, 2017
Revised: February 20, 2017
Accepted: March 12, 2017

Article in press: March 13, 2017
Published online: May 16, 2017

Abstract

Ticagrelor is a potent, direct P2Y12 antagonist with rapid onset of action and intense platelet inhibition, indicated in patients with acute coronary syndromes (ACS). This drug is usually well tolerated, but some patients experience serious adverse effects: Major bleeding; gastrointestinal disturbances; dyspnoea; ventricular pauses > 3 s. Given the unexpected high incidence of bradyarrhythmias, a PLATO substudy monitored this side effect, showing that ticagrelor was associated with an increase in the rate of sinus bradycardia and sinus arrest compared to clopidogrel. This side effect was usually transient, asymptomatic and not associated with higher incidence of severe atrioventricular (AV) block or pacemaker needs. A panel of experts from Food and Drug Administration did not consider bradyarrhythmias a serious problem in clinical practice and, accordingly, current labeling of the drug does not give any precaution or contraindication regarding this issue. However, recently some articles have described ACS patients with high-degree, life-threatening, AV block requiring drug discontinuation and, in some cases, pacemaker implantation. In this paper, we describe and discuss five published case reports of severe AV block following ticagrelor therapy and two other cases managed in our Hospital. The analysis of literature suggests that, although rarely, ticagrelor can be associated with life-threatening AV block. Caution and careful monitoring are required especially in patients with already compromised conduction system and/or treated with AV blocking agents. Future studies, with long-term rhythm monitoring, would help to define the outcome of patients at higher risk of developing this complication.

Key words: Ticagrelor; Atrioventricular block

Core tip: Ticagrelor is a potent, direct antiplatelet agent with rapid onset of action and intense platelet inhibition, indicated in patients with acute coronary syndromes (ACS). Even if well tolerated, some patients experience bradyarrhythmias complications, especially sinus bradycardia and sinus arrest. This effect is usually transient, asymptomatic and not associated with higher incidence of severe atrioventricular block. However, recent articles have described ACS patients with high-degree atrioventricular block requiring drug discontinuation and, in some cases, pacemaker implantation. In this paper, we describe and discuss five published reports and two other cases managed in our Hospital. We conclude that, although rarely, ticagrelor can be associated with life-threatening atrioventricular block. Caution and careful monitoring are required especially in patients with already compromised conduction system and/or treated with atrioventricular blocking agents. Future studies, with long-term rhythm monitoring, would help to define the outcome of patients at higher risk of developing this complication.

De Maria E, Borghi A, Modonesi L, Cappelli S. Ticagrelor therapy and atrioventricular block: Do we need to worry? *World J Clin Cases* 2017; 5(5): 178-182 Available from: URL: http://www.wjgnet.com/2307-8960/full/v5/i5/178.htm DOI: http://dx.doi.org/10.12998/wjcc.v5.i5.178

INTRODUCTION

Ticagrelor is a potent, direct P2Y12 antagonist with rapid onset of action and intense platelet inhibition. Unlike clopidogrel and prasugrel, it is not a thienopyridine and is not a prodrug. In patients with acute coronary syndromes (ACS) ticagrelor was superior to clopidogrel in reducing major adverse cardiac events and had a similar efficacy compared to prasugrel[1].

Ticagrelor is usually well tolerated, but some patients can experience serious adverse effects: Major bleeding (but rates are lower compared with other potent antiplatelet agents); gastrointestinal disturbances; dyspnoea; ventricular pauses > 3 s[1].

Given the unexpected high incidence of ventricular pauses in the landmark PLATO trial, this side effect was monitored by a prospectively designed, continuous electrocardiograph (ECG) monitoring substudy, including about 3000 patients[2]. In this study ticagrelor was associated with an increase in the rate of ventricular pauses > 3 s compared to clopidogrel (5.8% *vs* 3.6%, RR = 1.61, P = 0.01), mainly due to sinoatrial nodal pauses. This finding was only seen during the first week of therapy, while the incidence at 30 d was very low and similar between the two groups. Moreover, the great majority of pauses was asymptomatic and - even more important - there was no differences in the incidence of atrioventricular (AV) block or pacemaker need between groups[2].

As a consequence of this study[2] and after an "*ad hoc*" Food and Drug Administration meeting in 2011[3], a panel of experts concluded that the overall benefit of ticagrelor was superior to the risk of ventricular pauses, which appeared to be devoid of serious clinical consequences. Accordingly, current labeling of the drug does not give any precaution or contraindication regarding bradyarrhythmic effects.

CASE REPORT

Five published case reports of high-degree AV block after ticagrelor therapy

Recently, 5 reports of ACS patients in a "real world clinical scenario" have been published, describing cases of severe bradyarrhythmias due to AV block requiring intensive care, temporary pacing and sometimes the implant of a permanent pacemaker.

The first article was published by Goldberg *et al*[4] in 2015. A 52-year-old diabetic man with ACS and severe stenosis of ostial left anterior descending (LAD) artery underwent 2 bare metal stents implantation. Baseline ECG showed complete right bundle branch block (RBBB). Left ventricular ejection fraction (LVEF) was preserved. The patient, already taking bisoprolol 1.25 mg, was also treated with a loading dose of ticagrelor 180 mg. A few hours later, several episodes of paroxysmal AV block occurred, with pauses > 11 s and syncope, requiring the insertion of a temporary pacing system. Subsequently, bisoprolol was stopped and ticagrelor replaced with clopidogrel. After 3 d, the AV block resolved and temporary pacing was removed without implanting a permanent pacemaker. At 6 mo follow up, no AV block or other bradyarrhythmias were recorded.

Ünlü *et al*[5] reported about a patient who developed symptomatic Mobitz type Ⅱ AV block four days after receiving ticagrelor therapy in the context of ACS and left circumflex artery (LCA) stenting. The patient was already on beta-blocker therapy (bisoprolol 1.25 mg) before this acute event and baseline ECG showed first-degree AV block with narrow QRS. Ticagrelor and beta-blocker were withdrawn, but AV block still persisted after ten days, so a dual-chamber permanent pacemaker was implanted.

Goldberg *et al*[6] published the case of a 71-year-old female patient with ACS and proximal LAD occlusion, treated with thrombus aspiration and stent implantation. On ECG, she had complete left bundle branch block (LBBB) and was not taking beta-blockers. LVEF was moderately decreased. Ticagrelor was soon started, with recommended loading dose of 180 mg and continued with 90 mg twice a daily. Two days later, bisoprolol was started at 1.25 mg and after three hours complete AV block appeared, associated with sinus bradycardia, pauses up to 14 s and syncope. Temporary pacing was soon initiated, ticagrelor and bisoprolol were stopped. In two days AV block disappeared and temporary pacemaker was removed. A permanent pacemaker was not implanted and, at 6 mo follow up, no recurrence of AV

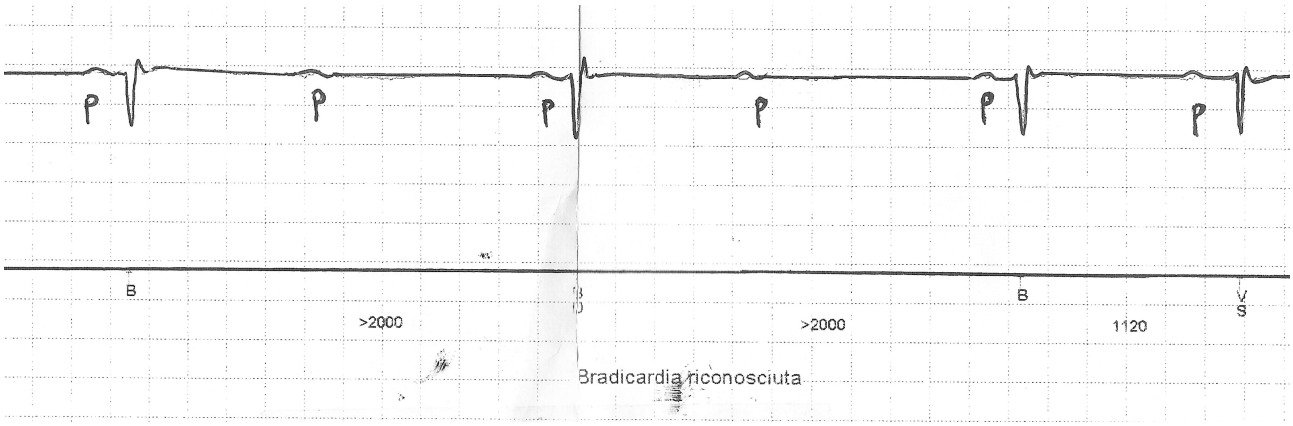

Figure 1 Patient #1. Continuous electrocardiograph monitoring showing paroxysmal episodes of 2:1 atrioventricular block with narrow QRS and lengthening of PP interval (associated sinus bradycardia).

block or other bradyarrhythmias were seen.

In the paper by Ozturk *et al*[7], a 62-year-old male diabetic patient (already on beta-blocker therapy) was admitted because of ACS and treated with right coronary artery (RCA) angioplasty. Baseline ECG showed first-degree AV block with narrow QRS. Seven hours after starting the 180 mg ticagrelor loading dose, a second-degree Mobitz Ⅱ type AV block appeared, associated with sinus bradycardia. The bradyarrhythmia was asymptomatic and well tolerated. Beta-blocker was stopped but AV block persisted up to seven days, so ticagrelor was replaced with prasugrel. On the third day after ticagrelor withdrawal, AV block disappeared. The patient was discharged and after one month he did not experience any other bradycardia.

Lastly, Baker *et al*[8] described a 56-year-old male diabetic patient with ACS and severe proximal LAD stenosis, treated with drug-eluting stent (DES) implantation. At baseline ECG PR interval and QRS complex were normal. One hour after starting ticagrelor loading dose, PR interval increased to 204 ms, so beta-blocker was not started. After additional three hours, the patient experienced nausea, diaphoresis and lightheadedness, with telemetry strip showing severe sinus bradycardia, sinus arrests and paroxysms of AV block. An emergent coronary angiography revealed a widely patent LAD stent and a temporary pacing system was inserted. Ticagrelor was discontinued and replaced with prasugrel; after 12 h bradyarrhytmias completely resolved. After some days, low dose beta-blocker was introduced and subsequent clinical course was uneventful.

Two further cases managed at our hospital

Here we describe two cases of ACS patients managed at our hospital, both with severe AV block following initiation of ticagrelor therapy.

The first was an 82-year-old male patient admitted with ACS and severe proximal LAD stenosis, who was treated with DES implantation and ticagrelor. He was already taking bisoprolol 1.25 mg. At baseline ECG PR interval was prolonged (about 280 ms) and QRS complex was narrow. A few days after discharge, the patient was admitted again because of several syncopal episodes without prodromes. Continuous ECG monitoring showed several paroxysmal episodes of 2:1 AV block associated with lengthening of PP interval (associated sinus bradycardia); these episodes persisted even after bisoprolol discontinuation (Figure 1), but did not require temporary pacing. It was decided to replace ticagrelor with clopidogrel: After some days AV block resolved, without the need of a pacemaker, and bradycardia did not recur over 6 mo follow up.

The second patient was a 76-year-old diabetic male with a recent DES implantation for LCA stenosis, in the setting of ACS hospitalization. Ticagrelor was started at usual doses just before angioplasty, while he was not taking beta-blocker because his baseline ECG displayed complete RBBB, left anterior hemiblock and a PR interval of 200 ms. Two weeks after starting ticagrelor, the patient was evaluated for recurrent syncopal episodes. A 24-h Holter ECG showed several episodes of paroxysmal complete AV block associated with PP interval lengthening (Figure 2). The patient was hospitalized and ticagrelor was replaced with prasugrel. During the following days, bradyarrhythmic phenomena were clearly reduced but did not completely disappear, so a permanent dual-chamber pacemaker was implanted.

DISCUSSION

The occurrence of ventricular pauses is a well-known side effect of ticagrelor, but it has been considered a transient phenomenon without serious clinical consequences. In this context, the most commonly reported arrhythmias are sinus bradycardia, sinus arrest and phases of junctional rhythm, usually fading away without symptoms. High-degree AV block occurred in a healthy volunteer after a large dose of the drug in a dose-finding study[1], but it was not considered a serious issue in the normal clinical setting[3]. It is only recently that some reports have described cases of high-degree, life-threatening, AV block requiring drug discontinuation[4-8], in patients with ACS.

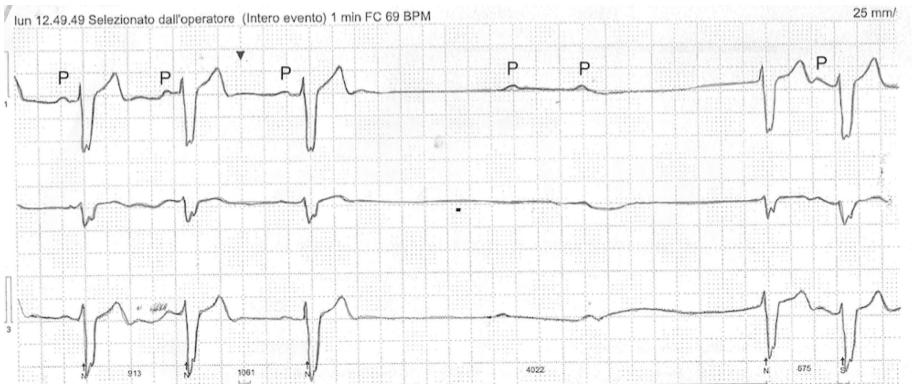

Figure 2 Patient #2. Holter electrocardiograph monitoring showing episodes of paroxysmal complete atrioventricular block associated with PP interval lengthening. Baseline wide QRS complex.

The exact mechanisms of bradyarrhythmic effect of ticagrelor, leading to AV block, are not fully clear. It has been hypothesized a direct effect of the drug on cardiac automaticity and conduction, but the most plausible explanation is the increase in adenosine plasma concentration due to the inhibition of its cellular uptake[9]. Adenosine has a potent AV blocking effect and also a negative influence on the activity of the sinoatrial node[1,3,9]. Almost all the patients, in the above-described reports, displayed AV block associated with sinus node inhibition, manifesting as sinus bradycardia (PP interval lengthening during the block) or sinus arrest.

A total of seven ACS patients with severe bradyarrhythmia have been described in this paper (including our two cases) and six of them presented at baseline with an already diseased conduction system (first-degree AV block, LBBB, RBBB), which is a known risk factor for developing high-degree AV block. The insertion of a temporary pacing system was necessary in three patients with severe clinical picture. A permanent pacemaker was implanted in two patients with persistent high-degree AV block (one with pre-existing long PR interval, the other with baseline RBBB + left anterior hemiblock). Moreover, five patients out seven were taking beta-blocker therapy, which obviously increases the risk of bradyarrhythmias.

It is interesting to note that four patients of this series suffered from diabetes and it has been reported that cardiac conduction abnormalities occur more frequently in diabetic patients[10], even subclinically. The patient described by Baker *et al*[8] had normal PR interval and QRS duration but he was a diabetic. It is unclear how many patients had pre-existing conduction system disease in PLATO trial, while diabetes was present in 25% of the population[8]. In the PLATO substudy investigating the incidence of bradyarrhytmias[2], the majority of patients with ventricular pauses were also taking beta-blocker therapy.

There are several reasons why ticagrelor can reasonably be considered the offending agent in this series of ACS patients: (1) high-degree AV block appeared briefly after the drug was started; (2) high-degree AV block disappeared (or improved) after its discontinuation; (3) not all patients were taking beta-blocker therapy and - when prescribed - doses were low; (4) AV block did not resolve after beta-blocker withdrawal; and (5) there was no other clear explanation for such an acute arrhythmic event and coronary lesions involved all major arteries.

These observations suggest that ticagrelor can have life-threatening, although rare, bradyarrhythmic effects in patients with ACS. Caution and careful monitoring are required especially in patients with already compromised conduction system and/or treated with AV blocking agents (even if these conditions are not currently considered as contraindications to ticagrelor therapy). Moreover, it remains to be established whether ticagrelor treated patients with more stable cardiovascular diseases (chronic stable coronary artery disease, peripheral artery disease)[11,12] or with cerebral ischemia[13] have a lower risk of bradyarrhythmias compared to ACS patients.

Future studies, with long-term rhythm monitoring, would help to define the outcome of patients at higher risk of developing this complication, including the potential association with diabetes and the risk of bradyarrhytmias in clinical settings other than acute coronary events.

COMMENTS

Case characteristics

Two patients with acute coronary syndrome were treated with ticagrelor and developed high-degree atrioventricular block; drug was discontinued but one patient required permanent pacing anyway.

Clinical diagnosis

Acute coronary syndrome and iatrogenic atrioventricular block.

Differential diagnosis

Primary atrioventricular block.

Laboratory diagnosis

Troponin elevation, all other blood exams were within normal limits.

Imaging diagnosis

Atrioventricular block at electrocardiograph.

Pathological diagnosis

Non-ST-elevation myocardial infarction.

Treatment
Drug discontinuation, pacemaker implant.

Related reports
Recent articles have described patients with acute coronary syndrome treated with ticagrelor who developed high-degree atrioventricular block requiring drug discontinuation and, in some cases, pacemaker implantation.

Term explanation
Acute coronary syndrome is a condition with myocardial ischemia due to acute coronary occlusion; high degree atrioventricular block is a life-threatening bradyarrhythmia due to impaired conduction of atrial impulses to the ventricles.

Experiences and lessons
Ticagrelor can have life-threatening, although rare, bradyarrhythmic effects in patients with acute coronary syndrome. Caution and careful monitoring are required especially in patients with already compromised conduction system and/or treated with atrioventricular blocking agents.

Peer-review
Comprehension and explanation of the problem is sound and the case-report is interesting.

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P- Reviewer: Coccheri S, Culic V, Nam GB, Ozdemir S
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Clinical Cases

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World J Clin Cases 2017 May 16; 5(5): 183-186

DOI: 10.12998/wjcc.v5.i5.183

ISSN 2307-8960 (online)

CASE REPORT

Unusual presentation of nasopharyngeal carcinoma with rectal metastasis

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Author contributions: Vogel M and Kourie HR initiated and wrote this case; Piccart M and Lalami Y reviewed and commented on this paper.

Institutional review board statement: The bordet institute's ethics committee provides a favorable opinion on the disclosure/publication of a patient clinical history to be reported as a case report.

Informed consent statement: The involved person in this case report gave his verbal informed consent prior to study and that was mentioned in the computerized medical file.

Conflict-of-interest statement: The authors confirm that they do not have any conflict of interest.

Manuscript source: Invited manuscript

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Received: December 6, 2016
Peer-review started: December 7, 2016
First decision: January 16, 2017
Revised: March 7, 2017
Accepted: March 23, 2017
Article in press: March 24, 2017
Published online: May 16, 2017

Abstract

Nasopharyngeal carcinoma (NPC) is a rare tumour that mainly metastasizes in lymph nodes, bones, lungs and liver. Colorectal metastases of NPC are extremely rare phenomenon and associated with a poor prognosis. We reported here a case of NPC with rectal metastasis, we discussed the treatment modalities and the prognosis after reviewing the similar cases described in the literature.

Key words: Nasopharyngeal carcinoma; Prognosis; Rectal metastasis; Treatment

Core tip: This is a rare case of nasopharyngeal carcinoma with rectal metastasis. After reporting the similar cases in the literature, we discussed the prognosis and the treatment of this rare phenomenon.

Vogel M, Kourie HR, Piccart M, Lalami Y. Unusual presentation of nasopharyngeal carcinoma with rectal metastasis. *World J Clin Cases* 2017; 5(5): 183-186 Available from: URL: http://www.wjgnet.com/2307-8960/full/v5/i5/183.htm DOI: http://dx.doi.org/10.12998/wjcc.v5.i5.183

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a head and neck cancer starting in the upper part of the throat, behind

Table 1 Three cases of colorectal metastases from nasopharngeal carcinoma

Ref.	Age (yr)	Sites of metastasis	Other metastasis	Colorectal metastases treatment	Follow-up
Lahuri *et al*[9] (2015)	61	Ascending colon	Right adrenal gland, supraclavicular lymph nodes, liver, lungs	Right hemicolectomy	Patient died 2 mo later
Suppiah *et al*[8] (2006)	64	Rectum	Abdominal lymph nodes	None	Patient died 15 d later
The present case	65	Rectum	Lung, adrenal glands, bones, lymph nodes, epiduritis, peritoneal carcinomatosis	None	Patient died 1 mo later

the nose (nasopharynx). This tumor has different distribution and incidence worldwide with endemic regions: The incidence of NPC is lower than 1/100000 in most countries; however, in the southern part of China (including Hong Kong), its incidence is higher and can reach 15 to 20/100000. Otherwise, the incidence of NPC is higher in males, the sex ratio being 2-3:1[1,2].

Genetic susceptibility, Epstein-Barr chronic virus infection, and environmental factors (*e.g.*, carcinogens and dietary factors) are risk factors associated to NPC[3,4]. NPC is divided into 3 subtypes by the World Health Organisation (WHO): Keratinizing squamous cell carcinoma, non-keratinizing carcinoma and undifferentiated carcinoma[5].

NPC has a tendency to metastasize to cervical lymph nodes, due to the abundant lymphatic network under the nasopharyngeal mucosa. At the time of diagnosis, 60%-85% of patients already have cervical metastasis[6]. The common distant metastasis are bones (65.9%), lungs (26.9%), liver (30.7%) and distant lymph nodes (28.5%). Other rare metastatic sites are described (2.4%) like spleen, kidney, pleura, breast gland, abdominal wall and thyroid gland[7]. The treatment of a non-metastatic patient is based on radiation therapy and/or chemotherapy. In metastatic NPC, the treatment is usually chemotherapy.

We report in this paper a rare presentation of NPC metastasizing to the rectum. We review the rare similar cases described in the literature about this association and discuss prognosis and treatment modalities of this unusual clinical presentation.

CASE REPORT

A 65-year-old smoker Caucasian patient presented to our department in July 2015 with stage IVc (T3N3bM1) non keratinizing undifferentiated NPC (WHO type Ⅲ). The diagnosis was established by computed tomography (CT) requested for the investigation of chronic nasal obstruction and multiple cervical nodes. The tumour measured 7.2 cm in diameter. Multiple lymph nodes were palpable in the supra clavicular fossa. Further investigations with a positron emission tomography-computed tomography (PET-CT) showed metastatic lesions in bones and lungs. The patient was treated with radiotherapy therapy, because he refused the Cisplatin-5FU chemotherapy regimen and bisphosphonates for his bone metastasis. A post radiotherapy PET-CT showed a moderate metabolic response of the nasopharyngeal tumour and cervical lymph nodes, but also a metabolic progression in the distant metastatic lesions. A close follow-up was advised. A new progression in the adrenal glands, Th10-Th11 epiduritis and peritoneal carcinomatosis were reported after 7 mo. Epiduritis was treated with radiation therapy.

A follow-up PET-CT, after one year of the diagnosis, showed a suspicious lesion in the rectum (Figure 1). Before including the patient into a phase I protocol, it was necessary to document this lesion. The work-up included a colonoscopy revealing a rectal mass, and a biopsy documenting a metastatic lesion from the well-known nasopharyngeal non-keratinizing undifferentiated carcinoma (Figure 2). It was decided to start a palliative chemotherapy but the patient died one month without receiving any treatment.

DISCUSSION

Rectum and colon metastases of NPC are extremely rare entities. To our knowledge, there are only two similar cases described in the literature: One with rectal metastasis[8] and another with colon metastases[9]. These lesions are usually asymptomatic and diagnosed on complementary imaging tests.

Thus, Two out of three patients were asymptomatic; the only symptomatic patient was reported by Suppiah *et al*[8] and presented with rectal bleeding and abdominal pain. In the reported cases the patients had multiple other metastases before the diagnosis of the colorectal metastases. In the 3 cases, the patient shortly died after the diagnosis. In the case reported by Lahuri *et al*[9], the metastasis was interpreted first as rectal adenocarcinoma, leading to a right hemicolectomy. Chemotherapy was planned but the patient died rapidly (Table 1).

Differentiating between a secondary lesion and another primary in front of a rectal lesion in the context of NPC is essential to guide therapy. The diagnosis cannot be confirmed without a pathological exam including immune-histochemical staining to further characterize the lesion. In case of a confirmed secondary lesion, systemic chemotherapy is indicated, while in case of a rectal primary, a loco-regional treatment is prioritized.

Usually, the treatment for non metastatic NPC at early stages is radiotherapy, including both sides

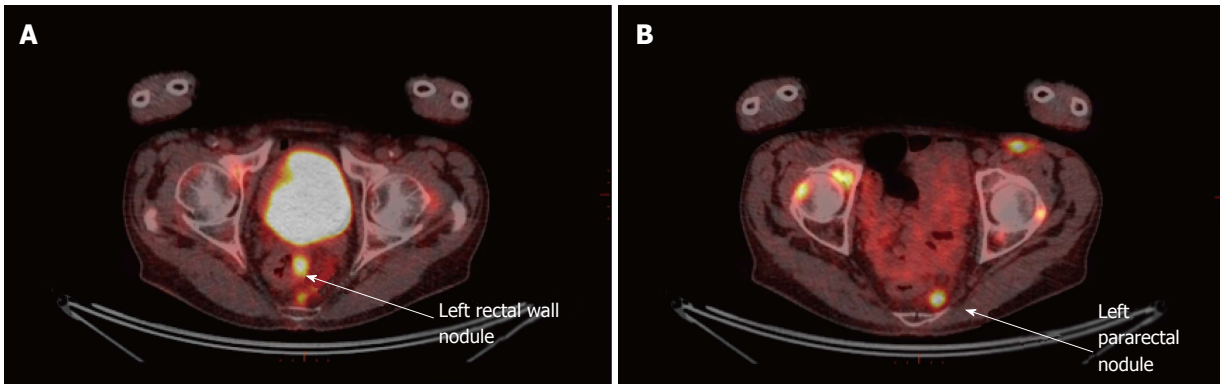

Figure 1 The positron emission tomography-computed tomography: Left rectal wall nodule (A) and left pararectal nodule (B).

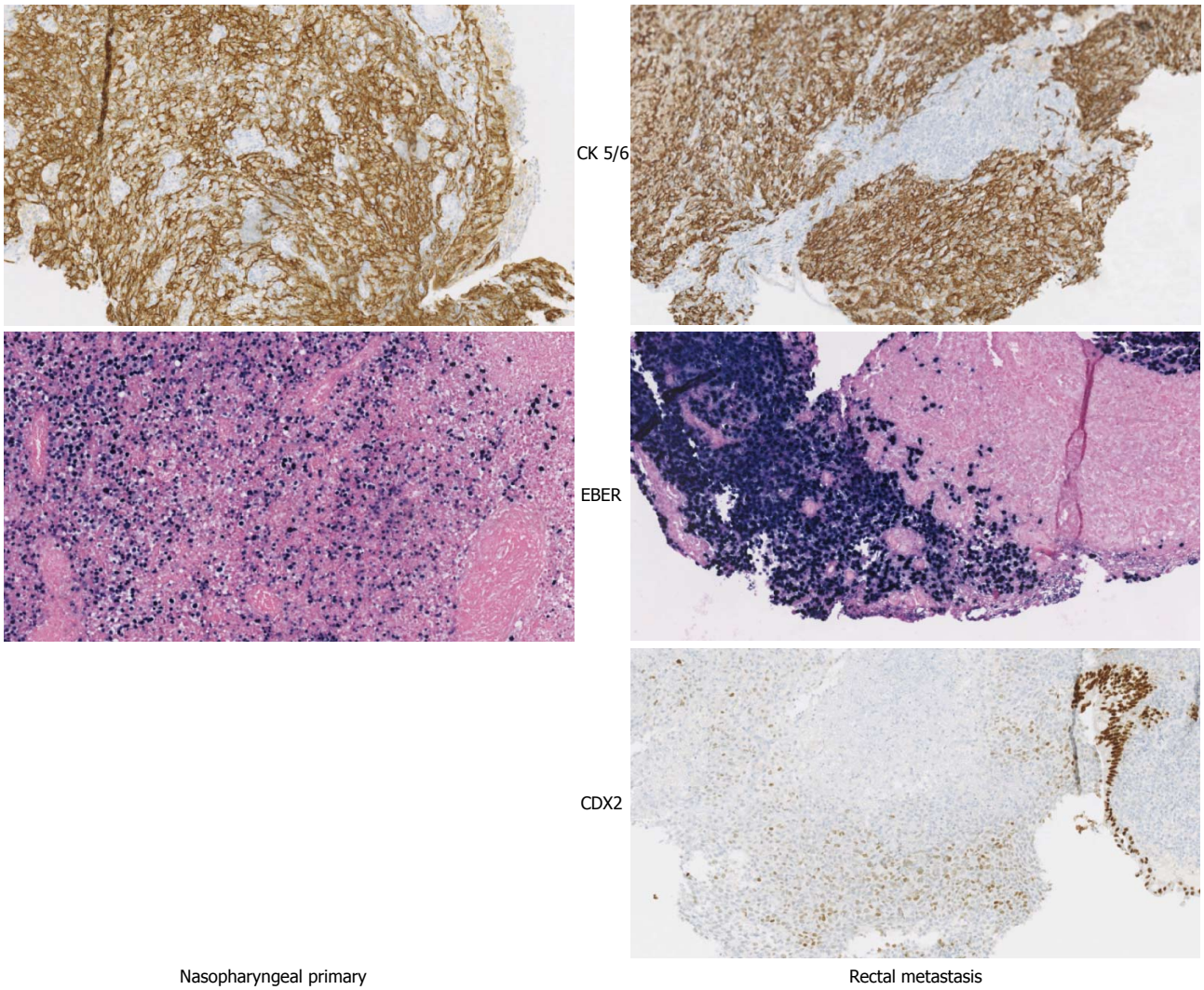

Figure 2 The nasopharyngeal primary and rectal metastasis are positive for CK 5/6 (confirming the epithelial origin) and EBER (confirming Epstein-Barr virus positivity). CDX2 the marker of colorectal origin is negative in the rectal lesion, confirming that it is a metastasis.

of the neck and retropharyngeal nodes. For locally advanced stages, the treatment guidelines advocate the combination of chemotherapy and radiotherapy. According to the response, surgery or brachytherapy can be considered as consolidation treatments[10]. In case of a metastatic NPC, the recommended first-line treatment is a platinum-based regimen and, more specifically, 5FU-cisplatin chemotherapy. In second line treatment, another chemotherapy can be proposed; the selection of which depends usually on the first-line treatment[11]. In the new era of checkpoint inhibitors, pembrolizumab, an anti-PD1 agent, showed remarkable

results in advanced multitreated NPC with response rates of 26% and disease control rate of 77%[12].

To conclude, the diagnosis of rectal metastases originated of NPC is necessary to orient the treatment modality and to determine the prognosis of the disease.

COMMENTS

Case characteristics

The patient did not present particular symptoms at the diagnosis of rectal metastasis of nasopharngeal carcinoma.

Clinical findings

The clinical examination of the patient was normal.

Differential diagnosis

A rectal primary adenocarcinoma was a possible differential diagnosis.

Laboratory findings

A moderate anemia was the only laboratory test abnormality.

Imaging diagnosis

A follow-up positron emission tomography-computed tomography, after one year of the diagnosis of pharyngeal adenocarcinoma, showed a suspicious lesion in the rectum.

Pathological diagnosis

The work-up included a colonoscopy revealing a rectal mass, and a biopsy documenting a metastatic lesion from the well-known nasopharyngeal non-keratinizing undifferentiated carcinoma.

Treatment

Palliative care was initiated because of the alteration of the performance status of the patient.

Experiences and lessons

It is very important to confirm the pathology of unusual localization of a suspicious lesion in a patient developing cancer to differentiate between a metastasis and a second primary. The prognosis and the treatment of a rectal metastasis of nasopharyngeal carcinoma and rectal primary is very different.

Peer-review

The manuscript is of interest and well written.

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P- Reviewer: Lai V, Li JJ, Tornesello ML **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Clinical Cases

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World J Clin Cases 2017 May 16; 5(5): 187-190

DOI: 10.12998/wjcc.v5.i5.187

ISSN 2307-8960 (online)

CASE REPORT

Elizabethkingia miricola: A rare non-fermenter causing urinary tract infection

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Author contributions: All authors have contributed equally to the acquisition of data, writing, drafting the article and revision of the manuscript; all the authors have given the final approval for the article to be published.

Institutional review board statement: The present case report has been retrospectively reviewed and reported and it didn't require any institutional ethics committee approval.

Informed consent statement: The patient involved in this study has given written informed consent authorizing use and disclosure of his health information.

Conflict-of-interest statement: None.

Manuscript source: Invited manuscript

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Received: November 16, 2016
Peer-review started: November 21, 2016
First decision: January 14, 2017
Revised: February 9, 2017
Accepted: March 12, 2017
Article in press: March 13, 2017
Published online: May 16, 2017

Abstract

Elizabethkingia miricola (*E. miricola*) is a gram-negative non-fermentative bacterium which is rarely encountered. It is usually misidentified or considered as a contaminant in routine microbiology laboratories due to the limitations in conventional biochemical techniques. However, with the advent of the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS), the identification of non-fermenters has become easy and this has led to enhanced understanding of the clinical significance of these uncommonly isolated microorganisms. The genus *Elizabethkingia* has only two species *E. meningoseptica* and *E. miricola*. Both of these organisms are known to be multi-drug resistant and therefore, their accurate identification and antimicrobial susceptibility testing are necessary prior to the initiation of appropriate therapy. In the world literature till date, only 3 cases of sepsis caused by *E. miricola* have been reported. We present the first case of *E. miricola* association with urinary tract infection.

Key words: *Elizabethkingia miricola*; Antibiotics; Urinary tract infections; Matrix-assisted laser desorption ionization time-of-flight; Non-fermenters

Core tip: Non-fermenters except *Pseudomonas* and *Acinetobacter* are less commonly associated with urinary tract infection (UTI). But recently an upsurge in a number of reported cases has been noted due to the use of MALDI-TOF which is an easy and reliable identification technique. Till date in literature, there is no reported case of *Elizabethkingia miricola* (*E. miricola*) causing UTI, although its significance in blood and sputum samples of sepsis patients has been demonstrated earlier. This is the first case report showing a clinical association of *E. miricola* with symptomatic UTI and also demonstrating the multidrug resistance nature of this organism.

Gupta P, Zaman K, Mohan B, Taneja N. *Elizabethkingia miricola*: A rare non-fermenter causing urinary tract infection. *World J Clin Cases* 2017; 5(5): 187-190 Available from: URL: http://www.wjgnet.com/2307-8960/full/v5/i5/187.htm DOI: http://dx.doi.org/10.12998/wjcc.v5.i5.187

INTRODUCTION

Urinary tract infections (UTI) are amongst the most common bacterial infections occurring in human beings during their lifetime[1]. The usual organisms responsible for UTI belong to the family *Enterobacteriaceae* and gram-positive bacteria like *Staphylococcus* and *Enterococcus*[2]. UTI caused by non-fermenters (NF) is being increasingly reported especially in the nosocomial settings, with *Pseudomonas and Acinetobacter spp.* being the most common agents. However, UTI due to other NFs like *Alcaligenes, Flavobacterium, Oligella, Flavimonas, Agrobacter, Weeksiella* are also on the rise[3]. Routine laboratory identification of NF is difficult and labour-intensive, which often misclassifies or misidentifies these agents and thereby may mask the exact clinical significance of these isolates. Nowadays, the identification of these NF has become easy by the advent of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). We recently encountered a case of UTI caused by rare multidrug resistant non-fermenter *E. miricola*, which was identified by MALDI-TOF.

CASE REPORT

A 25-year-old female presented with complaints of increased bowel frequency, oliguria, fever and abdominal pain since one month. Detailed history revealed that the patient had difficulty in micturition for past two weeks. The routine laboratory investigations revealed a haemoglobin of 7.8 gm/dL, total leucocytes count 3200 cells/mm^3, platelet count of 70000 cells/mm^3. Renal function tests revealed normal sodium concentration (139 mEq/L), hyperkalemia (8.2 mEq/L), hyperuricemia (74 mg/dL) and elevated creatinine levels (7.5 mg/dL). Coagulation profile was normal. Ultrasonography (USG) revealed bilateral hydroureteronephrosis with normal renal parenchyma and features of vesicoureteric reflux. The midstream urine sample was subjected to microbiological testing. The wet mount microscopic examination showed 1-2 RBCs, numerous pus cells and bacteria per high-power field[4]. The semi-quantitative culture done on the cysteine lysine electrolyte deficient agar showed significant bacterial growth (colony count > 10^5 CFU/mL). The colonies were non-lactose fermenting, translucent, greenish blue, smooth having entire edges and became mucoid on prolonged incubation. Subculture on MacConkey agar showed pale, translucent, glistening colonies with entire edges (Figure 1). Gram staining showed 0.5 μm × 2 μm gram-negative bacilli, with no spores and no capsule. The isolate was also subjected to conventional identification using a battery of biochemical tests. The isolate was catalase positive, oxidase positive, produced indole, was non-nitrate reducing, mannitol fermenting, esculin and gelatinase hydrolysis positive. Urease was produced and this test helped to distinguish it from *E. meningoseptica*. The isolate was confirmed as *Elizabethkingia miricola* (*E. miricola*) (identification score of 2.29) by using MALDI-TOF-MS (BrukerDaltonics, Bremen, Germany). The antimicrobial susceptibility was carried out using Kirby-Bauer disc diffusion method and the antibiotics tested were chosen from the available literature as there are no CLSI guidelines available till now[5,6]. The isolate was sensitive to gentamicin, ceftriaxone, aztreonam, piperacillin-tazobactam and imipenem, and resistant to ampicillin, ciprofloxacin, levofloxacin, vancomycin and colistin. The patient was started on piperacillin-tazobactam and responded well to the treatment. The patient improved clinically and the follow-up urine culture after two weeks of therapy was sterile.

DISCUSSION

E. miricola was first isolated from Mir space station, Russia and hence named as *E. miricola*[7]. Previously, it was classified into genus *Chryseobacterium* but later in 2005, the genus was changed to *Elizabethkingia* on the basis of the comparative analytical studies involving DNA hybridization and sequencing of the 16S rRNA region[8]. *E. miricola* is a gram-negative (0.5 μm × 1-2.5 μm), non-motile, non-spore-forming bacterium. It grows well on blood and MacConkey agar producing non-fermenting sticky colonies. Biochemical reactions show indole positive, citrate positive, produce acid from D-glucose, D-fructose, D-lactose, trehalose, D-mannitol, D-maltose, but not from D-xylose, L-arabinose, D-cellobiose, sucrose and raffinose. It can be differentiated from *Chryseobacterium* because of the absence of yellow pigment in culture. Urease production is the test used to differentiate *E. miricola* from *E. meningoseptica*[8]. Till date, *E. miricola* has been isolated from blood and sputum and has been found to be responsible for sepsis. The first case of *E. miricola* was reported in 2008 in an adult with mantle cell carcinoma, who underwent stem cell transplant[5]. In this case, *E. miricola* was isolated from sputum and blood and the identification was confirmed using 16S rRNA sequencing. Later on, *E. miricola* was isolated from the blood sample of a young female with alcoholic pancreatitis[6]. More recently, *E. miricola* has been isolated from a patient with severe sepsis and pulmonary abscess[9]. In both the above cases, the isolate was identified by MALDI-TOF. In the present case, *E. miricola* was isolated from the urine sample of a young female with clinical features of UTI and bilateral hydroureteronephrosis. The clinical presentation pointed towards differential diagnosis like pyelonephritis, renal abscess, renal infarction, venous obstruction or ATN. However, the USG findings of bilateral hydroureteronephrosis and sterile blood culture pointed

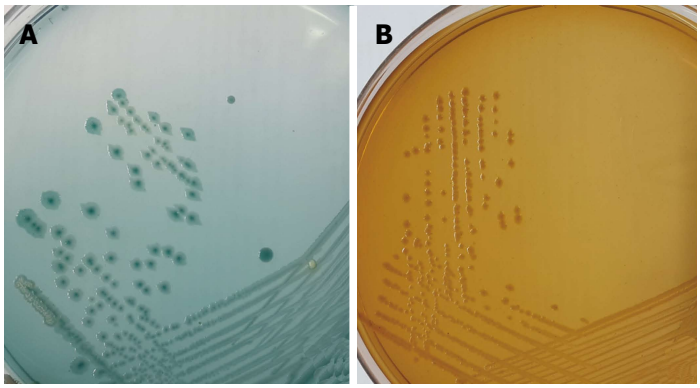

Figure 1 Culture plates showing the growth of *Elizabethkingia miricola* on (A) cystein-lactose-electrolyte-deficient medium agar and (B) MacConkey agar.

towards localised urinary tract infection.

E. miricola has been found to be multidrug resistant similar to *E. meningoseptica* which is known to harbor β-lactamases showing resistant to β-lactams and carbapenems[10]. The *E. miricola* isolates have been found to be resistant to many antibiotics. Previous studies have shown resistance to ampicillin, ceftazidime, imipenem, gentamicin, cotrimoxazole, colistin and with variable susceptibility to ciprofloxacin, vancomycin and rifampicin[5,6,11]. It is interesting to note that, *E. miricola* isolates in previous studies were sensitive to levofloxacin, but in our case, the isolate was resistant to both ciprofloxacin and levofloxacin. Limited clinical reports, varied susceptibility profiles, lack of antimicrobial susceptibility breakpoint and no defined consensus for the empiric treatment regimen makes it difficult to treat such rare organisms.

We present the first case report of human UTI caused by rare multidrug resistant *E. miricola*. The present case emphasizes the clinical importance of rare non-fermenters like *E. miricola* in human infections especially in case of UTI. The knowledge of newer species and their antimicrobial susceptibility profile will help in formulating appropriate antibiotic treatment regimens to tackle such rarely encountered bacteria.

COMMENTS

Case characteristics

A 25-year-old female complaining of difficulty in micturition, oliguria fever with abdominal pain.

Clinical diagnosis

Urinary tract infections (UTI) with bilateral hydroureteronephrosis.

Differential diagnosis

Chronic pyelonephritis.

Laboratory diagnosis

The routine laboratory investigations revealed anemia, leucopenia, hyperkalemia, hyperuricaemia and elevated creatinine levels. Urine culture had significant bacterial growth (colony count >10^5 CFU/mL) of *Elizabethkingia miricola* (*E. miricola*).

Imaging diagnosis

Bilateral hydroureteronephrosis.

Pathological diagnosis

Bilateral hydroureteronephrosis with urinary tract infection.

Treatment

Piperacillin-tazobactam.

Related reports

E. miricola has been reported to cause sepsis and pulmonary infection.

Experiences and lessons

Rare non-fermenters can cause UTI and prompt identification is required to guide proper antimicrobial therapy. CLSI/EUCAST guidelines need to be developed.

Peer-review

Interesting case of unusual bacterial cause of UTI with a severe clinical scenario.

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P- Reviewer: Simone G, Woo HH **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

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