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Contents

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Contents

World Journal of Clinical Oncology
Volume 8 Number 5 October 10, 2017

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REVIEW

Further the liquid biopsy: Gathering pieces of the puzzle of genometastasis theory

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Abstract

Metastasis is the major cause of mortality in cancer disease and still constitutes one of the most controversial mechanism, not yet fully understood. What is almost beyond doubt is that circulatory system is crucial for cancer propagation. Regarding this system, much attention has been recently paid to liquid biopsy. This technique is aimed to detect circulating tumor cells (CTCs) and circulating nucleic acids so it can be used as a tool for diagnostic, prognostic and follow-up of patients. Whereas CTCs tend to be scarce in serum and plasma from cancer patient, abundant circulating nucleic acids can be detected in the same location. This fact, together with the genetic origin of cancer, stands out the relevance of circulating nucleic acids and shed light into the role of nucleic acids as drivers of metastasis, a recently discovered phenomenon called Genometastasis. This innovative theory supports the transfer of oncogenes from cancer cells to normal and susceptible cells located in distant target organs through circulatory system. What is more, many biological processes haven been described to deliver and secrete circulating nucleic acids into the circulation which can allow such horizontal transfer of oncogenes. In this review, we focus not only on these mechanisms but also we demonstrate its putative role in cancer propagation and give insights about possible therapeutic strategies based on this theory. Our objective is to demonstrate how findings about cell-to-cell communications and previous results can agree with this unprecedented theory.

Key words: Genometastasis; Cancer metastasis; Circulating Nucleic acids: Circulating tumor cells; Liquid biopsy; Exosomes; Virtosomes

Core tip: Liquid biopsy not only constitutes a promising tool for cancer diagnostic and patient follow-up but also it may help in the comprehension of metastasis. This technique has revealed how circulating tumor cells are limited in blood, while circulating nucleic acids are much more abundant. This property, together with the demonstrated capability of circulating nucleic acids to transform susceptible cells, strongly support the theory of genometastasis. This theory sustains that cancer propagation relies on gene transfer from malignant cells to normal cells. We pretend to gather all these concepts, also including cell-to-cell communication mechanisms to demonstrate this phenomenon.

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CONCEPT OF LIQUID BIOPSY

Liquid biopsy

Traditionally, tissue biopsy has been used to diagnose and manage diseases. In cancer, biopsies are used to determine histological properties of the tumor as well as its genetic profile for diagnostic, prognostic purposes and prediction of response to therapies. However, the characteristic heterogeneity of tumors makes it necessary to analyze different parts of the same tissue which results in repeated sampling. Obtaining several tissue biopsies involves a high risk for the patient as well as economic cost for the system. As an alternative to tissue biopsy, liquid biopsy constitutes a promising and less invasive technique.

Liquid biopsy consists on the detection of cancer-derived molecular biomarkers, such as tumor cells or cell-free nucleic acids (cfNA) in biological fluids, mainly in blood. Given the non-invasiveness properties of the technique, it is possible to take repeated samples and so, to follow the progression and evolution of the disease in contrast to the static image from tissue biopsy.

The effectiveness of this approach has been demonstrated in different malignancies including breast, pancreatic and colorectal cancer (CRC)[1]. In the case of pancreatic cancer, liquid biopsy provides an advantageous technology regarding the anatomical and clinical difficulties for pancreatic tissue[2]. It would also help in the early detection of this disease, which is usually diagnosed at an advanced stage because it develops with no symptoms. For its part, CRC is mainly characterized by its heterogeneous genetic profile, in which new mutations constantly appear during tumor development[3]. These new mutations may confer proliferative capacities to tumor cells and, thus, molecular and genetic analysis of the whole tumor might be crucial during CRC follow-up. Similarly, tumor genotyping is also required in the case of anti-EGFR therapies, to which only the patients with KRAS wild-type gene respond. Thus, liquid biopsy can be conceived not only for recording tumor progression but also for selecting the most suitable treatment.

As mentioned before, liquid biopsy can be intended to detect circulating tumor cells (CTCs) and/or circulating cfNA.

CTCs

CTCs can be secreted into circulation by primary and metastatic tumor deposits. In 1869, during autopsy of a breast cancer patient, CTCs were first identified as cells similar to those of the primary tumor, presented in the bloodstream[4]. These cells are mainly found in patients with malignant diseases like carcinomas, being extremely rare in healthy subjects and patients with nonmalignant diseases[5].

CTCs can be difficult to obtain given its heterogeneous morphology and its limited amount in the circulation: They constitute one cell per 1×10^9 normal bloodstream cells in patients with metastatic cancer[6]. In other terms, in 7.5 mL of blood from metastatic carcinoma patients, only 5 to 50 CTCs are presented on average[7]. This small cell number makes it difficult to detect CTCs, especially small subpopulations of tumor cells, which can harbor crucial mutation for tumorigenesis. However, many attempts and approaches have been designed to isolate CTCs. Most of them are based on antibody identification of cell surface markers, such as EpCAM, or size differences between CTCs and the rest of blood cells[8]. Once CTCs are obtained, they have to be further analyzed through genome sequencing. Nevertheless, these isolating techniques might not provide the whole spectrum of CTCs, uncovering the tumor heterogeneity. As an example, basal-like breast cancer CTCs with low levels of EpCAM may not be captured using this cell surface marker determinant[7]. On the other hand, false-positive CTC results can also be found in the case of patients with benign inflammatory disease such as Crohn disease. It has been shown that 11% to 19% of these patients present small numbers of circulating epithelial cells detectable that can be confused with CTCs[9]. In addition, although correlation between cell number and disease severity have been established in metastatic patients from breast, colon and prostate cancer[10-12], less is known about early-stage tumors and CTC number. Altogether, more studies are required to elucidate the relationship between tumor burden and the number of CTCs in order to verify the clinical utility of CTCs as prognostic markers[7].

It is also worth noting that CTCs are difficult to grow in culture, which questions the functionality of these cells. Thus, it can be hypothesized that these cells are more likely to constitute death cells, poured by tumor mass, than active cells responsible for metastasis emergence.

Circulating nucleic acids

Regarding to circulating nucleic acids we can make a distinction between circulating cell-free DNA (cfDNA) and cell-free RNA (cfRNA).

Circulating cfDNA: The first association between cancer and the presence of circulating cfDNA was established in 1977 by Leon *et al*[13] who detected a higher concentration of DNA in serum from cancer patients. Ten years later, Stroun *et al*[14] confirmed this relation by isolating and characterizing DNA obtained from the plasma of cancer patients. Moreover, it was further shown that patients with malignant tumors have higher circulating cfDNA levels than patients suffering benign disease[15]. The tumor origin of such cfDNA was also confirmed by the identification of tumor-specific abnormalities such as loss of heterozygosity (LOH) of microsatellites and methylation of CpG islands[16,17]. In addition from tumor cells, plasma cfDNA may come from blood cells and other tissue-specific cells. However, the proportion of DNA derived from different origins widely varies. In fact, circulating tumor DNA proportion range between 0.01% and 93% in cancer patients[7,18].

cfDNA is usually found in plasma as with a double-stranded structure, although single-stranded circulating DNA has also been identified[19-21]. It should be noted that DNA molecule need to be protected by different complexes or other molecules, described in detail below, in order to avoid its degradation by serum nucleases.

Circulating cfRNA: Circulating cfRNA was first isolated in 1987 from serum of patients with malignant disorders and culture media of different malignant cell lines. It was initially found in the form of RNA-proteolipid complex[22]. As it happens with DNA, it is no surprising to detect cfRNA associated with other molecules since it alone can be very labile due to the increased amounts of RNases present in the circulation.

Circulating RNA consist of messenger RNA (mRNA) and microRNA (miRNA). Regarding to mRNA, different transcripts have been identified to be overexpressed in plasma of tumor patients, especially human transcriptase reverse telomerase (hTERT) mRNA levels in malignancies such as breast cancer or colon cancer[23-25]. miRNA molecules are fragments of 19-25 bp non-coding RNA molecules which derive from 70-100 bp hairpin precursor molecules. By posttranscriptional regulation, they modulate the expression of target genes involved in many physiological and pathological process such as development, cell proliferation, differentiation or apoptosis[26,27].

Circulating nucleic acids as biomarkers: Although the term nucleic acids refers to both types of molecules, special attention has been paid to cfDNA in the field of liquid biopsy because it carries the tumor-associated mutations and thus, it represents an attractive biomarker.

As commented before, circulating DNA gives more detailed information about the heterogeneity of the tumour because it may come from different cells with presumably different genomic alterations, which can be detected by sequencing.

Likewise, circulating cfDNA is much easier to isolate than CTCs because it is abundantly present in blood, especially in patients with advanced disease[28]. Indeed, circulating DNA extraction can be performed following a simple protocol that does not exceed 5 h[18]. Once it is isolated, PCR, followed by DNA sequencing can be used to detect tumor-specific genetic aberrations which may also help in the comprehension of tumor dynamics. In this issue, droplet digital PCR, together with genome-wide high throughput sequencing, provide a high sensitivity and specificity for detecting mutations[29]. These new tools for DNA analysis are also contributing to give a more profound insight into the presence and role of circulating DNA, among its value as a biomarker.

CTCs and circulating cfDNA

It would be reasonable to suspect that tumor cfDNA found in the circulation can be released by CTCs. However, the discrepancy between the number of CTCs and the quantity of circulating DNA discards this theory. Considering the average amount of circulating DNA in a ml of plasma from advanced-stage cancer patients (17 ng) and the amount of DNA contained by a single human cells (6 pg), more than 2000 CTCs would be required if CTCs were the primary source of circulating DNA. Conversely, less than 10 CTCs per 7.5 mL of blood are found on average[18]. Therefore, tumor cfDNA might come from different regions within the tumor and thus, it may better represent tumor genetic heterogeneity. This fact, together with its high concentration in blood, suggests that circulating DNA might be a better liquid biopsy-derived biomarker. In the following section, we will focus on the reasons why DNA can be released into circulation.

WHERE DO CIRCULATING NUCLEIC ACIDS COMES FROM AND HOW DO THEY CIRCULATE IN THE BLOOD STREAM

Depending on how they are released, circulating nucleic acids can be found in different forms including molecular or macromolecular complex, linked to serum proteins or internalized in vesicles such as exosomes or microvesicles (Figure 1). In general terms, circulating nucleic acids can be either passively released, by apoptotic and necrotic cells, or actively released by living cells.

Passive release

During cell-death mechanisms, such as necrosis or apoptosis, both circulating DNA and circulating RNA can be liberated into bloodstream by dying or dead cells.

In necrosis, cellular DNA is incompletely and nonspecifically digested. In this condition, a smearing pattern

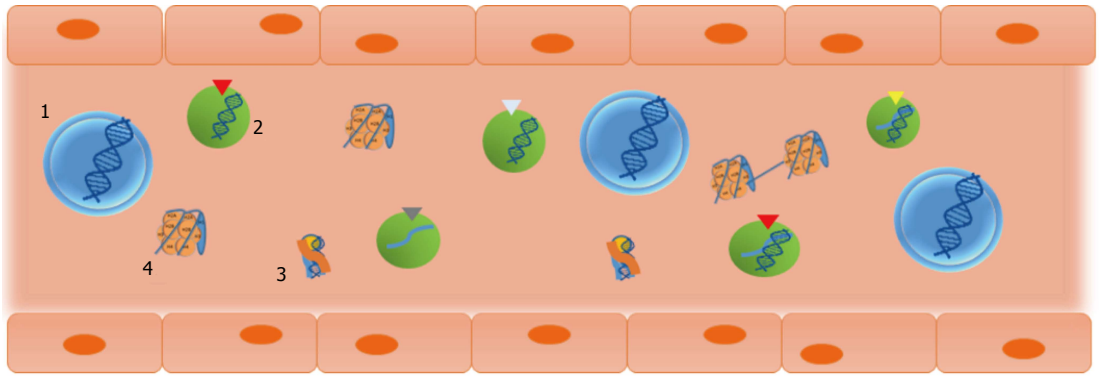

Figure 1 Circulating nucleic acids can be present in different forms. If actively released, circulating nucleic acids can be found inserted in exosomes (1) and microvesicles (2), or associated with RNA and lipoproteins forming a complex called the virosome (3). Circulating nucleic acids can be also passively released, mainly through apoptosis, in the form of oligo- or mono-nucleosome (4).

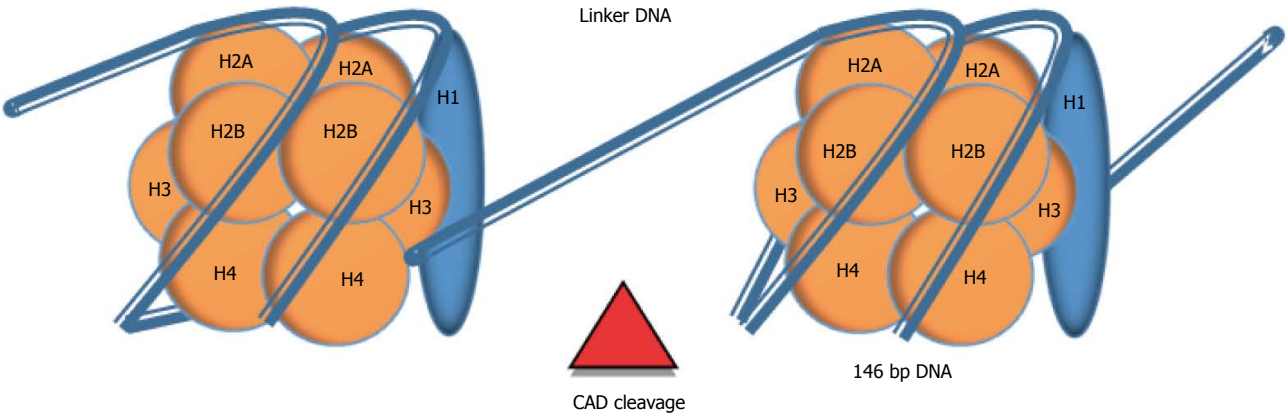

Figure 2 Chromatin cleavage during apoptosis can be a source of circulating DNA. Circulating DNA can be found in the form of nucleosomes. Each nucleosome is composed of 147 of DNA wrapped around an octamer of histones (H2A, H2B, H3 and H4). An extra histone (H1) stabilizes this complex. During apoptosis, CAD enzyme cleaves in the naked DNA that links each nucleosome (DNA linker), releasing oligo- or mono-nucleosomes. In cancer, where a higher cellular turnover is required, this process can be overloaded and nucleosomes can be secreted into circulation. CAD: Caspase-activated deoxyribonuclease.

would be observed when DNA is run electrophoretically in agarose gel. However, when circulating DNA is analyzed by agarose-gel electrophoresis, a ladder pattern is observed. This feature indicates that necrosis is not the major source of circulating DNA although it may be a possible contributor given the presence of DNA fragments ranging from 21 kb to 80 kb in length in blood plasma samples[14,30].

The mentioned ladder pattern is formed by fragments ranging from 180 bp to 1000 bp which matches with the fragments released from chromatin cleavage into nucleosomes, a process that occurs during apoptosis[30].

Nucleosome: Nucleosomes are molecular complexes that allow DNA stabilization and packing into the nucleus. In each nucleosome, 146 bp of double-stranded DNA are wound on an octamer of positively-charged proteins called histones (H2A, H2B, H3 and H4), through electrostatic interaction. Nucleosomes are linked by 10 to 100 bp of naked DNA, termed as linker DNA. An extra histone (H1), which is localized outside the octamer, stabilizes the tertiary structure of the chromatin chain[31] (Figure 2). Cell death by apoptosis implies the activation of a set of caspases that catalyze the hydrolysis of cellular components. Some of these caspases (*e.g.*, Caspase-3) trigger the activation of endonucleases, especially the caspase-activated deoxyribonuclease (CAD). Endonucleases cleavage chromatin through linker DNA, the most accessible region, generating oligo- and mono-nucleosomes that are packed into vesicles called apoptotic bodies. Apoptotic bodies are subsequently released from the cells and phagocytosed by macrophages and dendritic cells. Nevertheless, in conditions when higher cellular turnover is required, such as inflammation or tumor cell proliferation, this process collapses and nucleosomes are liberated into circulation[30,31]. Then, cell-free nucleosomes can be internalized into cells by crossing plasma membrane and penetrating into the nucleus from where it can alter gene expression[32].

It is worth noting that the octamer of histones of the nucleosome protects DNA molecule from its degradation by circulating endonucleases. It also should be noted that tumor-derived circulating DNA may be more fragmented than DNA derived from healthy cells as recent publications have shown[33].

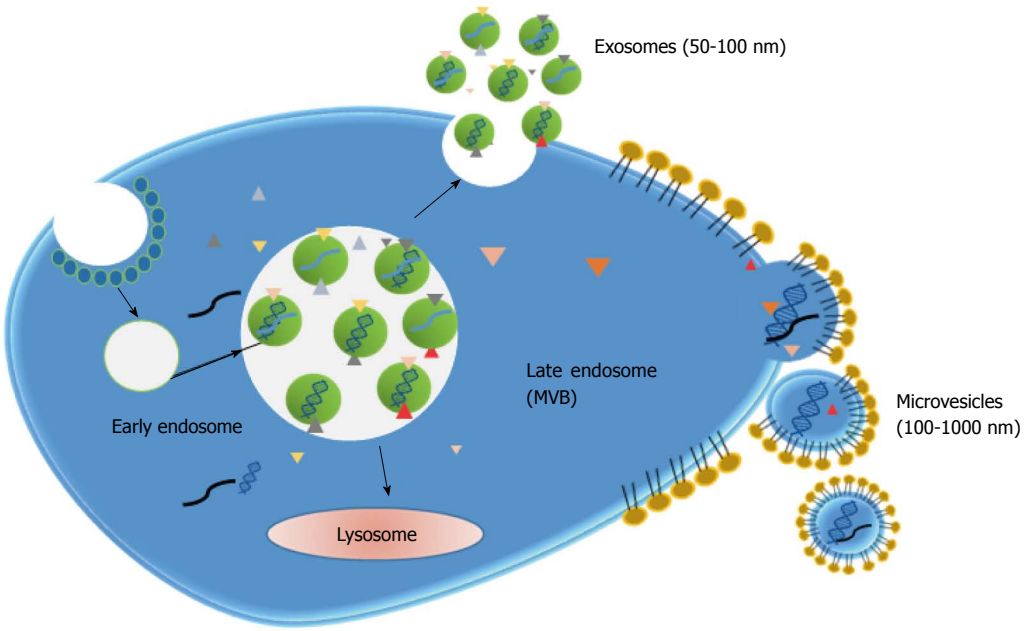

Figure 3 Exosomes and microvesicles can harbour nucleic acids. Exosomes and microvesicles are generated by different pathways. Exosomes derive from the recycling endosomal pathway, in which the late endosomes (MVB) merge with the plasma membrane instead of with the lysosome, releasing the exosomes. Exosomes encapsulate cytoplasm material such as proteins, RNA or DNA. Microvesicles result from plasma membrane budding, containing cellular components from both cell membrane and cell cytoplasm, including nucleic acids. MVB: Multivesicular bodies.

The apoptotic origin is confirmed by the existence of circulating mitochondrial DNA (mitDNA). In contrast to nuclear DNA, mitDNA is a circular and smaller (16.5 kb) molecule of DNA, not protected with histones[34]. It can be secreted to the circulation during cell death (*e.g.,* apoptosis) and mitophagy, which consist on the elimination of damaged mitochondria through autophagy[35]. Due to its elevated copy number, circulating mitDNA may account for a high proportion of the total circulating DNA found in blood. It can be present in circulation in both protein-associated and free form[34]. Circulating mitDNA measurement and mutation analysis has been proposed to diagnose different malignancies such as breast tumors or epithelial ovarian cancer and hepatocellular or colorectal cancer, respectively[1,36].

Active release

In addition to passive secretion, circulating nucleic acids can be actively released through cell-derived vesicles, such as exosomes and microvesicles, from living cells. The phenomenon of spontaneously released DNA was first described in lymphocytes, frog auricles and cultured cell lines[37-43]. Like nucleosomes, vesicles protect cell-free nucleic acids from the circulating nucleases and hinder the recognition by the immune system[32].

Exosomes: Exosomes are small lipid membrane vesicles (50-100 nm) secreted from various cell types including dendritic cells, B cells, T cells, tumor cells and epithelial cells[44]. Exosomes result from the recycling endosomal pathway. During endocytosis, vesicles are generated at the plasma membrane and enter into the cell forming early endosomes. These early endosomes are transformed into late endosomes which then develop multivesicular bodies (MVB). MVBs can fused with lysosomes for degradation of its content or with the plasma membrane. In this last case, internal vesicles are liberated into the extracellular space and termed exosomes[45] (Figure 3). Therefore, exosomes contain membrane and cytoplasmic components such as lipids, proteins and RNA (mainly mRNA and miRNA). Additionally, the presence of single-stranded and double-stranded DNA was further demonstrated[46].

Furthermore, exosomes are capable to enter in recipient cells by either binding to cell surface receptors through adhesion molecules or being internalized through mechanism similar to endocytosis and so can act as cellular communicators. What is more, these vesicles can travel to distant sites of the organism and release the packed biomolecules into local and remote cells. Exosomes can bear different proteins including transmembrane proteins, such as major histocompatibility complex (MHC), and other intraluminal proteins and oncoproteins such as mutant KRAS[47]. Proteins delivered by exosomes can activate or inhibit different signalling pathways, altering cell function. For its part, exosomes-derived miRNA can modulate gene expression by posttranscriptional regulation.

Particularly in cancer cells, exosomes secretion is usually increased. Tumor-derived (TD) exosomes may favour tumor growth by inhibiting apoptosis and increasing cellular proliferation. As an example, it was demonstrated that exosomes increased cellular proliferation in gastric cancer cell lines by activating Akt phosphorylation[48]. Moreover, it has been described that

TD exosomes can also facilitate cancer invasion and metastasis by regulating stromal cells, remodelling the extracellular matrix and stimulating angiogenesis[47,49].

Regarding to nucleic acids, the presence of mRNAs, miRNAs and DNA highlights the role of exosomes as carriers of genetic information too. Indeed, the role of exosome-derived miRNA has been widely demonstrated. Depending on its target gene, miRNA can act either as a tumor suppressor or as a tumor enhancer. For instance, miR-198 has been demonstrated to be released by T-lymphoblast exosomes performing a tumor suppressor role in lung, liver and colorectal cancer[50-55]. Conversely, other miRNAs favour tumor progression such as miR-21, which can also be secreted through exosomes[56,57]. It should be considered that exosomes generally carry more than one kind of miRNA, so its effects depend on the combination of miRNAs presented[58].

Microvesicles: Microvesicles emerge from plasma membrane budding and the following fission of the vesicles from the plasma membrane. They have a larger size (100-1000 nm) than exosomes and membrane composition is more similar to that of plasma membrane than exosome membrane composition (Figure 3). Thus, tumor-derived microvesicles constitute a representation of the tumor proteomic signature. Microvesicles can be secreted by different cell types including hematopoietic cells, endothelial cells, mesenchymal stem cells and cancer cells[59]. It has to be taken into consideration that, despite their differences, the terms exosomes and microvesicles are usually interchanged. Moreover, in most studies, vesicles are obtained by approaches that cannot discriminate both types of vesicles and so it may be difficult to classify published information according to each type.

As well as exosomes, microvesicles are key elements in cell-to cell communication, modulating the recipient cell phenotype. For instance, it has been shown that cultured hematopoietic progenitor cells can be reprogrammed by microvesicles derived from embryonic stem cells. In fact, these microvesicles contained mRNA for several pluripotent transcription factors demonstrating an additional mechanism of horizontal transfer of genetic material[60].

In cancer scenario, microvesicles from tumor and non-tumor cells can also be secreted to transfer miRNA and other oncogenic proteins to facilitate invasion and tumor growth. Likewise, it was reported that tumor-derived microvesicles carrying surface determinants of tumor cells, like chemokine receptors, and mRNA for growth factors, such as vascular endothelial growth factor (VEGF) or hepatocyte growth factor (HGF), were able to internalize in monocytes and so, change its phenotype and biology activity[61]. Furthermore, it was also published that tumor-associated macrophages can secrete microvesicles containing miRNA that can promote breast tumor cell invasiveness[62].

Virtosomes: The existence of virtosomes was first described by Stroun and Gahan[63]. The virtosome is a macromolecular complex formed by newly synthesized DNA and RNA associated with lipoproteins, which is spontaneously released from living cells. To form this structure, newly DNA is synthesised in the nucleus and then transferred to the cytosol. In cytosol, DNA associates with a lipoprotein, which serves as a protector from nuclease digestion, and before leaving the cell, an RNA molecule is attached to the complex. The complex can exit the cell in an energy-dependent way and entering other cells by mechanism not well understood[63].

Viral nucleic acids: Viral DNA as well as viral RNA can be found in plasma and serum from patients[30]. Given the relation between some viral infections and particular malignancies, detection of viral DNA might be used as a biomarker for certain neoplastic disease. As an example, cell-free DNA from Epstein-Barr virus (EBV) serves as diagnostic and prognostic marker for nasopharyngeal carcinoma[64].

Nucleic acids can be attached to cell surface

cfDNA and RNA can also be found attached to the exterior part of the plasma membrane from where they can be detached and released into circulation. DNA is usually found in the cell surface of leucocytes and erythrocytes and can be internalized by receptor recognition or remain associated with the surface[30].

GATHERING PIECES

Liquid biopsy has been commonly proposed as a tool for cancer diagnostic, characterisation and prognostic in patients as both CTCs and cfNA provide relevant information from the tumor. Nonetheless, very much attention has been paid for this practical application without taking full account of the possible biological roles of cfNA in blood. Although it is known how circulating nucleic acids can be presented in blood (as it has been described), its function in this location is still controversial. Considering the above commented discrepancy between CTCs number and cfDNA quantity as well as the active mechanisms of cfNA release, cfNA presence in blood does not appear to be a mere coincidence. What is more, many evidences point to cfNA as a key driver of metastasis, which is the essence of the theory of genometastasis.

GENOMETASTASIS: A PUTATIVE MECHANISM INVOLVED IN THE ORIGIN OF METASTASIS

Metastasis is an enormously complex process that remains to be a major problem in the management of

cancer. The metastatic properties of tumor cells were extensively investigated from 1970s, although so much earlier (as soon as 1889) it was proposed the "seed an soil" theory that today is still alive and even under constant reformulation (*e.g.,*[65])

During the seventies, some theories were proposed, such as that most primary tumor cells have a low metastatic potential, and that during later stages of tumorigenesis rare cells acquire metastatic capacity through additional somatic mutations (reviewed in[66]). This suggested mechanism had contrary evidence in other studies that concluded that metastases are a random representation of disseminated tumor cells, all of which have the ability to form a metastasis[66]. On the whole, it might be said that the discussion of "dynamic heterogeneity" models *vs* "clonal dominance" theories prevailed during two decades, always under the premise of a circulatory view of cancer progression. In fact, nowadays, many authors appears to not conceive any other way, as showed in the recent literature, *e.g.,* "Metastasis is the consequence of a cancer cell that disperses from the primary tumor, travels throughout the body, and invades and colonizes a distant site"[65].

This view does not explain some questions such as the lack of correlation between the sites of development of metastasis and the anatomic vascular filters[67]. Several million cells per gram of tumor can be shed daily into the lymphatic system or bloodstream. However, insufficient data exists to quantify the fraction of shed tumor cells that successfully seed secondary tissues. Moreover, the fate of blood borne tumor cells is controversial and many experimental evidence are contradictory: Whereas in some models most circulating cells die, in others most survive and extravasate. Nevertheless, all studies show that most cells entering the vasculature fail to form macroscopic foci at distant sites (reviewed in[68]). On the other hand, an unquestionable fact is that the identification and characterization of CTC require extremely sensitive and specific analytical methods, much more than detection of cell-free tumor DNA.

In connection with the circulatory theory, surgical maneuvers for tumor resection (particularly, gastrointestinal tumors) have classically been designed to avoid blood dissemination of cancer cells, which hypothetically results in a lower risk of recurrence and metastasis (reviewed in[69]). However, benefits of such procedures have not been fully demonstrated yet. At late 1990s, our group challenged that technical axiom-not sufficiently supported-, and performed a study in colorectal cancer patients that showed that the use of no-touch isolation techniques in colorectal cancer was not justified, based on lack of evidence indicating the detachment of cells from the tumor at surgery[70]. Apart from the clinical discussion (which has not been finished yet), the fact was that circulation of tumor cells appeared to have lesser value than attributed. In parallel, the evidence of high levels of cell-free nucleic acids in plasma of cancer patients and tumor-bearing animals led us to examine the biological role of such molecules[1,29,71]. Firstly in cancer models using immunocompetent animals and later in clinical studies with colon cancer patients, we demonstrated the biological feasibility of gene transfer and of the transformation of cells by cell-free tumor-derived nucleic acids in the plasma[41,72,73]. In the light of such results, we proposed that cell-free nucleic acids in the plasma participate in tumorigenesis and the development of metastases *via* transfection-like uptake of such nucleic acids by susceptible cells. This putative phenomenon was named as "genometastasis" (Figure 4).

Albeit, at first, some authors exhibited more criticism than enthusiasm for this hypothesis[74], later experimental evidence supported the existence of the genometastasis. Moreover, the assays that substantiated this theory were repeated and enlarged by other authors, who confirmed our results[75-78].

Consistently with our theory, recently Mittra *et al*[79] have asserted that circulating nucleic acids, far from being biologically inert particles, have significant deleterious functions in the host. According with their results, they concluded that circulating nucleic acids are ubiquitous and continuously arising, and freely can enter healthy cells integrate into their genomes, inflicting repeated damage to the somatic DNA. Moreover, the authors have suggested that the somatic genome may not be stable, but rather remains in a state of turmoil characterized by dsDNA breaks, genomic instability and apoptosis affected by integration of circulating DNA. These events may lead to deletions, duplications and rearrangements causing DNA mosaicism[79]. Once demonstrated the existence of this phenomena and connecting all previous results, it would be even naive to think that progression of cancer is not related to triggering genetic events and consequent genomic rearrangements.

Nonetheless, despite the soundness of results, some authors were still showing their reticence to accept the genometastasis as a feasible mechanism for metastasis, arguing mainly that such theory is not able to explain the tropism of metastasis[80]. In our opinion, this is an erroneous assessment perhaps motivated by a partial view of the phenomenon that we described, because, precisely, in both own and other authors' studies, it was shown that not all kind of cells were transformed by plasma[73,75,76]. Our model is, not only incompatible with the idea of specific tropism for metastasis, but it is really proper to search tropism mechanism.

Mittra *et al*[79] clearly demonstrated that cellular/nuclear uptake of DNA is energy dependent and requires an active metabolic machinery of the recipient cells, which might be a first selection. However, it is possible that the key is not only in the characteristics of susceptible cells ("soil"), but also in the particles circulating in the transforming plasma ("seed"). In fact, there is an increasing stream of studies about the potential of extracellular vesicles on induction of cellular transformation and most of those observations are fully

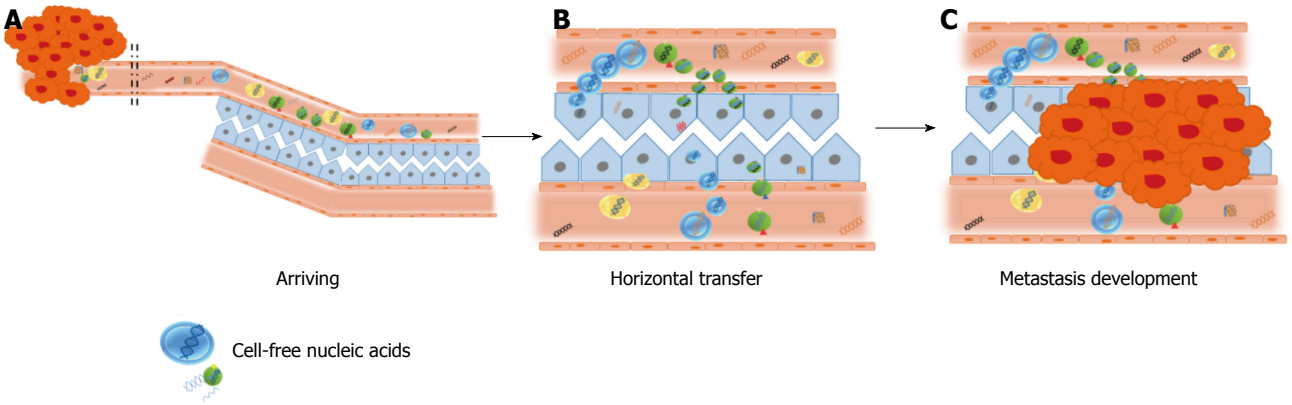

Figure 4 The theory of genometastasis. The putative mechanism of genometastasis: A: Releasing of nucleic acids to the blood stream; B: Transfection of susceptible cells; C: Malignization of transfected cells.

consistent with the theory of genometastasis[81-83].

LOOK TO THE FUTURE: TREATMENTS BASED ON THE GENOMETASTASIS PHENOMENON

Traditionally, treatments directed to prevent metastasis have been based on the use of cytotoxic substances that avoid circulation, homing and reproduction of malignant cells. If we assume that circulating nucleic acids in cancer patients have a role in the production of metastasis, a new scenario can be opened up. We can imagine a variety of strategies for interfering with these circulating nucleic acids either during their travel or during the horizontal transfer at the target organ.

Perhaps, the most immediate approach appears to be the use of enzymes to degrade circulating nucleic acids. The idea of enzymes-based therapies for cancer hovered since four decades ago[84], and in the last years, some convincing approaches have been reported. For example, Trejo-Becerril *et al*[85] have reported that systemic treatment with DNAse I and a protease mix in rats decreased DNA and proteins from serum and had antitumor effects. Interestingly, Patutina *et al*[86] have reported that tumor-bearing animals treated with RNase A and DNase I had a general systemic and immunomodulatory effect that led to a drastic suppression of metastasis development. Undoubtedly, those results support the role of the genometastasis phenomenon in the development of metastasis and encourage deepening.

Other potential therapeutic approach might be based on the use of potentially transfecting particles charged by "good sequences" of nucleic acids. It has not been enough tested but, theoretically, it is possible that such particles promote a "competitive" effect with cell-free tumor nucleic acids and, then, avoiding metastasis. In this line, virtosomes (*i.e.*, the mentioned DNA-RNA-lipoprotein complex) might constitute a useful tool. These particles are spontaneously released from healthy human, other mammalian, avian, amphibian and plant cells in a regulated and energy-dependent manner[63]. Likewise, these released virtosomes have been demonstrated to enter other cells[87-89]. More importantly, the biology of the recipient cells may be also modified if virtosomes come from a different cell type. Experiments with virtosomes in an immunocompetent animal model of colorectal cancer, showed a virtosomal effect in blocking cell multiplication in both *in vitro* and *in vivo* studies, resulting in a scape from inhibition at times after inhibition initiation. These results could indicate the existence of a response derived from the initiation of an immune reaction[90].

In other way, some previous studies have suggested the possibility of silencing these circulating oncogenic signals through RNA interference. As an example, the use of some micro-RNA can determine a novel regulatory pathway in KRAS-driven cancers, which offers a potential therapeutic target for their eradication[91], if this microRNAs are harboured by particles such as virtosomes or exosomes.

Indeed, a lot of strategies can be suggested in order to interfere with the horizontal transfer mechanism, responsible for the transformation of healthy and normal cell into malignant cell. Nonetheless, as happens with all new paradigms, lots of further lines of research are required in this field.

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MINIREVIEWS

Stereotactic radiotherapy for prostate cancer: A review and future directions

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Abstract

Prostate cancer affects over 200000 men annually in the United States alone. The role of conventionally fractionated external beam radiation therapy (RT) is well established as a treatment option for eligible prostate cancer patients; however, the use of stereotactic body radiotherapy (SBRT) in this setting is less well defined. Within the past decade, there have been a number of studies investigating the feasibility of SBRT as a potential treatment option for prostate cancer patients. SBRT has been well studied in other disease sites, and the shortened treatment course would allow for greater convenience for patients. There may also be implications for toxicity as well as disease control. In this review we present a number of prospective and retrospective trials of SBRT in the treatment of prostate cancer. We focus on factors such as biochemical progression-free survival, prostate specific antigen (PSA) response, and toxicity in order to compare SBRT to established treatment modalities. We also discuss future steps that the clinical community can take to further explore this new treatment approach. We conclude that initial studies examining the use of SBRT in the treatment of prostate cancer have demonstrated impressive rates of biochemical recurrence-free survival and PSA response, while maintaining a relatively favorable acute toxicity profile, though long-term follow-up is needed.

Key words: Stereotactic body radiotherapy; Prostate cancer; Radiation therapy; Hypofractionation; Toxicity; Stereotactic ablative radiotherapy

Core tip: Initial studies examining the use of stereotactic body radiotherapy (SBRT) in the treatment of prostate cancer have demonstrated impressive rates of biochemical recurrence-free survival and prostate specific antigen response, while maintaining a relatively favorable acute toxicity profile. Here we review a number of recent

prospective and retrospective studies to evaluate the efficacy and toxicity of SBRT in the treatment of low, intermediate, and high-grade prostate cancer.

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INTRODUCTION

According to the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database there were 220800 new cases of prostate cancer diagnosed in 2015 and an estimated 27540 deaths[1]. Since the advent of routine prostate specific antigen (PSA) screening, the majority of cases are confined to the prostate and radiation therapy (RT) is often employed as an alternative to surgical resection. Currently, the National Comprehensive Cancer Network guidelines recommend a combination of observation, radical prostatectomy, conventionally fractionated external beam RT, and androgen deprivation therapy (ADT), depending on stage and risk profile. Stereotactic body radiation therapy (SBRT), which entails five or fewer fractions of at least 5 Gray (Gy), is not currently included in the national guidelines.

A number of studies have evaluated the efficacy of conventionally fractionated external beam RT. With follow up ranging from 5 to 20 years and total doses ranging from 78 to 86 Gy, reported biochemical control was greater than 80% for the favorable risk group compared to approximately 60% for the high risk group. Total dose was also a factor as biochemical control was approximately 60% at lower doses and greater than 80% for higher doses with an estimated overall risk reduction of 40%-50% with respect to biochemical failure[2-5]. This review will examine the evidence for SBRT in comparison to conventional fractionation in the era of modern treatment, and the future direction of SBRT in the treatment of prostate cancer.

Stereotactic radiosurgery has already been applied with great success in other types of cancer, most notably malignancies of the lung and brain (*i.e.,* stereotactic radiosurgery). In the case of lung malignancies, SBRT offers an overall survival benefit as compared with conventionally fractionated RT and offers an alternative when patients are not surgical candidates. Recent work has sought to extend SBRT to prostate cancer with the goal of demonstrating improved outcomes. However, as described above, the threshold for proving non-inferiority is high given excellent results with conventionally fractionated radiation therapy, surgery, or even observation in low risk patients[6].

In this review we present trials of SBRT in the treatment of prostate cancer. Data from these studies are relatively immature with a maximum median follow-up time of 60 mo. Since overall survival at 60 mo or less is expected to be high even in the absence of intervention, we focus on factors such as biochemical recurrence-free survival (bRFS), PSA response, and toxicity. Here we attempt to provide a balanced perspective on the benefits and challenges associated with the use of SBRT in the treatment of prostate cancer.

RESEARCH

Studies included in this review were identified by performing a search of existing literature appearing in the PubMed database, using the keywords "prostate" and "SBRT", which returned a total of 270 results. To qualify for inclusion, treatments must have been delivered in five fractions or fewer, with the exception of one study that employed SBRT as a boost upon conclusion of a conventionally fractionated course. Both prospective and retrospective studies were included. In addition, only those studies that provide detailed results for both PSA response and toxicity were considered for inclusion. Computed tomography and/or magnetic resonance imaging were used for treatment planning in all studies, and treatment positioning was achieved with either daily or real-time imaging. A total of 14 studies met these criteria and are presented here. The remaining 256 published works were excluded for a variety of reasons, including: Insufficient follow-up, lack of toxicity data, or irrelevance to the topics addressed in this review.

SBRT AS A DEFINITIVE THERAPY IN PROSTATE CANCER

SBRT is currently an evolving treatment approach, with no established standard fractionation schedule. There have been a number of single-institution experiences reported with promising results that show local control rates comparable to conventional fractionation, albeit with a much shorter length of follow-up. While hypofractionated radiation therapy has been used in the treatment of prostate cancer since the 1960's, it has historically been undertaken with 2D planning, as described in Lloyd-Davies *et al*[7]. The emergence of advanced technologies, such as intensity modulated radiation therapy (IMRT) and image guided radiation therapy (IGRT), have greatly improved toxicity. However, this review of 209 patients treated with a six-fraction regimen over three weeks established the feasibility of hypofractionation with good local control and an absence of significant morbidity[7].

Among the earliest published studies, Madsen *et al*[8] reported initial findings from their SHARP trial in which forty enrolled patients were treated with five fractions of 6.7 Gy. The authors assumed an alpha/beta ratio of 1.5, similar to other prostate SBRT studies, resulting in a biologically equivalent dose of 78 Gy.

However, the advantage is that hypofractionated dose prescriptions produce an acute effect profile consistent with a significantly lower conventionally fractionated prescription. Enrolled patients were all categorized as low-risk with combined Gleason scores of six or less. All patients achieved a PSA nadir below 2.0 ng/mL and thirteen achieved a nadir below 0.5 ng/mL. There were three biochemical failures resulting in a bRFS rate of 90% at 48 mo. The group also reported an acute toxicity profile comparable to a conventionally fractionated trial conducted at the Cleveland Clinic[9]. The five-year follow-up shows an overall survival of 75% with no prostate cancer related deaths and a resolution of all GU and GI toxicities; however, 50% of the twenty-six patients who were potent at the time of treatment subsequently became impotent[10]. The median PSA nadir was 0.65 ng/mL at a median time of 24 mo.

Building upon past studies utilizing HDR brachytherapy as a monotherapy, King *et al*[11] enrolled 67 low- to favorable intermediate-risk patients in their phase Ⅱ trial. All participants were treated in 5 fractions of 7.25 Gy[12]. They report a four-year bRFS rate of 94% and a median PSA of 0.5 ng/mL at follow-up. There were, however, two biopsy proven failures, but neither of these patients were found to have metastatic disease. Furthermore, patients tolerated the treatment relatively well; there were no grade 3 or higher rectal toxicities, and the grade 3 urinary toxicity rate was 3.5% with no grade 4 urinary toxicities. The toxicity profile compared favorably to past conventionally fractionated dose-escalation and hypofractionated studies. The authors attribute this, in part, to the relatively narrow expansion margins that SBRT affords (in this study, 5 mm overall and 3 mm posteriorly). One unique feature of this trial is that the first twenty-two patients were treated QD (*i.e.,* five consecutive days) while the balance were treated every other day (QOD). Interestingly, the QOD cohort experienced fewer grade 1 to 2 urinary and rectal toxicities, with no change in the rate of grade 3 urinary toxicity.

Boike *et al*[13] conducted a multicenter dose escalation study, enrolling a total of forty-five stage T1-2 patients with Gleason scores of seven or less. Their dose prescriptions were based upon prior nude mouse xenograft studies and radiobiologic modeling of established high dose rate (HDR) brachytherapy[14]. Patients were divided into three cohorts, each of which was treated in 5 fractions of 9, 9.5, or 10 Gy. The study began with the 9 Gy cohort and a ninety day observation period was enforced to evaluate for acute toxicity before the subsequent higher dose cohort was treated. PSA response was favorable in all cohorts with an overall mean nadir of less than 0.4 ng/mL. The authors were particularly focused on evaluating the toxicity associated with this protocol, as comparable preceding studies limited the total dose to 36.25 Gy or less[15,16]. Acute toxicity was generally limited, with only grade 1 or 2 symptoms reported. A limited number of higher-grade late toxicities arose as follows: One case of a grade 4 rectal ulcer, and one case each of grade 3 cystitis and dysuria.

Hannan *et al*[17] report the five-year follow-up results of Boike *et al*[13] and add a phase Ⅱ portion consisting of 47 patients treated to 50 Gy in 5 fractions. This study achieved a remarkable collective five-year bRFS rate of 98.6%, which the authors acknowledge may be overestimated due to their follow-up protocol. This rate exceeded those previously reported by groups that employed other modalities, including: Intensity modulated radiation therapy, hypofractionated radiation therapy, and radical prostatectomy. The majority of acute grade 2 toxicities and all late grade 3 to 4 toxicities occurred in the 50 Gy arm. Three out of a total of four grade 4 toxicity events affected the rectum. Though the stoppage criteria for severe toxicity were not met, the authors ultimately concluded that doses less than 50 Gy are advisable.

Katz *et al*[18] recruited 304 low, intermediate, and high-risk patients. Expansion margins of 5 mm overall and 3 mm posteriorly were employed and patients were treated with five fractions of either 7 or 7.25 Gy. No acute grade 3 or 4 toxicity was reported, and of the 48 patients who reached the twelve-month follow-up at the time of publication, only one late grade 3 toxicity occurred. Quality of life (QOL) was measured using the Expanded Prostate Cancer Index Composite (EPIC) questionnaire. Patients reported an initial decrease in bowel and urinary QOL, but returned to baseline. However, sexual QOL decreased by approximately 10% and remained at that level. By twelve months, 28% of patients achieved a PSA nadir of less than 0.5 ng/mL. A total of four individuals failed biochemically. Long term follow-up revealed a seven-year biochemical disease free survival of 95.6%, 89.3%, and 68.5% for low, intermediate and high-risk cases, respectively[19]. Minimal late toxicity was reported.

Jabbari *et al*[20] treated 20 low- or favorable intermediate-risk patients with four fractions of 9.5 Gy while another 18 intermediate- and high-risk patients were treated with EBRT and ADT combined with an SBRT boost consisting of two fractions of 9.5 Gy. Four patients received an integrated 1 Gy/fraction boost to the dominant intraprostatic lesion. Treatment was planned so as to mimic HDR brachytherapy in terms of dose heterogeneity and outside-of-target sparing. No acute grade 3 or higher toxicity was observed and two patients experienced late grade 3 toxicities. With a median follow-up of 18.3 mo, the median PSA nadir for the monotherapy group was 0.47 ng/mL and 0.10 ng/mL for the combined therapy group. No patients experienced biochemical failure at the time of publication. Though the results are generally favorable, the authors caution that additional accrual and follow-up is needed to ensure durable relapse-free survival. Bolzicco *et al*[21] treated the spectrum of low- to high-risk patients and also stratified PSA response based upon ADT use. The authors note a trend towards lower nadirs with the addition of ADT (median nadir of 0.62

ng/mL *vs* 0.18 ng/mL at 3 years), though statistical significance was not reported. Oliai *et al*[22] undertook a dose escalation trial for low- to high-risk patients and also stratified PSA response by ADT use, reporting a mean PSA nadir that decreased from 0.4 ng/mL to less than 0.1 mg/mL with the addition of ADT.

The Naples and Stanford groups compiled a combined cohort of 41 patients with a median follow-up time of 5 years[23]. The Stanford patients were treated with 5 fractions of 7.25 Gy and the Naples patients were treated with 5 fractions of 7 Gy. The reported five-year biochemical progression free survival rate was 92.7% with a mean PSA nadir of 0.35 ng/mL, though the Stanford subset had a mean nadir of 0.18 ng/mL, significantly lower than entire the cohort average. There were three biopsy proven failures. Treatment was generally well tolerated, though acute toxicities were not explicitly reported. There was one reported case of late grade 3 toxicity and no late grade 4 toxicities.

McBride *et al*[24] reported on 45 patients who received 5 fractions of 7.25 to 7.5 Gy. Biochemical progression free survival at three years was reported as 97.7% and the median PSA nadir at twelve months was 0.91 ng/mL. One late grade 3 urinary obstruction and two late grade 3 proctitis events were noted. There was a statistically significant decrease in the Sexual Health in Men (SHIM) survey score, along with the EPIC bowel and sexual function scores. All three of the reported grade 3 toxicities resolved with corrective intervention.

In 2013, the American Society for Radiation Oncology (ASTRO) released a policy statement supporting the use of SBRT as an appropriate alternative to conventional RT for low- to intermediate-risk disease. This allowed researchers to begin focusing attention on addressing specific technical challenges associated with SBRT. Mantz *et al*[25] tried to control for prostate movement with the implementation of reliable organ tracking techniques to ensure adequate dose localization and to minimize toxicity to surrounding sensitive tissues. Towards this end, they enrolled 102 low-risk patients who were subsequently treated using a proprietary technology, the Calypso® System (Varian Medical Systems, Palo Alto, CA, United States) that uses implanted transponders to track the prostate in real-time during treatment. Patients are then treated on conventional linear accelerators. Other studies used a competing real-time tracking platform, CyberKnife® (Accuray, Inc., Sunnyvale CA), which is comprised of a 6 MV linear accelerator mounted to a robotic arm. Patients received five fractions of 8 Gy, and achieved a mean PSA of 0.27 ng/mL at 24 mo. The toxicity profile was among the best of the prostate SBRT studies with no grade 2 or higher rectal events and only two grade 3 urinary events, both acute. Twelve-month EPIC scores showed a return to near-baseline after an initial decline. These results suggest that real-time tracking may provide a means of reducing toxicity without compromising efficacy.

Recently, efforts have been made to expand the use of SBRT in the treatment of intermediate- and high-risk prostate cancer. Anwar *et al*[26] built upon the previously discussed Katz study, delivering a two-fraction boost of either 9.5 or 10.5 Gy total to 50 patients who had already received a course of conventionally fractionated EBRT to doses of 45-50 Gy. The reported five-year bRFS for all patients was 83%, with a median PSA nadir of 0.05 ng/mL achieved at a median time of 26.2 mo. No grade 3 or higher toxicity was noted. Four cases of disease progression were recorded, all of which occurred outside of the field of radiation. By comparison, a multi-institutional analysis found five-year bRFS rates of 84% and 81% for intermediate- and high-risk patients, respectively[27]. The results of this work compare favorably to HDR boost therapy, suggesting that SBRT boost may be a viable option for intermediate- and high-risk prostate cancer patients. Additionally, this work showed that SBRT resulted in an increased rate of PSA decline as compared to conventionally fractionated EBRT, which is a feature associated with improved clinical outcomes.

Davis *et al*[28] analyzed outcomes for a total of 437 localized prostate cancer patients treated with SBRT at one of seventeen centers in the United States and Australia. Patients were enrolled between 2006 and 2015 and all risk categories were represented. Two-year bRFS was found to be 99.0%, 94.5%, and 89.8% for low, intermediate and high-risk groups, respectively. Higher Gleason score was associated significantly with lower biochemical disease-free survival. Fifteen patients experienced biochemical failure. In general, the SBRT treatments were well tolerated; no patients experienced high-grade genitourinary or gastrointestinal toxicity. The authors corroborated an assertion others had made that SBRT does induce a rapid twelve-month decline in PSA, as observed across multiple studies. A similar pooled analysis by King *et al*[27] that included 1100 patients treated at eight institutions from 2003 to 2013 found collective bRFS rates of 95%, 84% and 81% for low-, intermediate- and high-risk patients, respectively. Patients were treated to a total dose of 35 to 40 Gy over five fractions. Biochemical failure at a median follow-up time of 36 mo was low, at 4.5%, and a subset of these patients were determined to have a PSA bounce that subsequently declined. Interestingly, neither total dose nor the use of ADT had a statistically significant effect on bRFS. The authors conclude that SBRT compares favorably to other definitive treatments and should be considered as an alternative therapy in low- and intermediate-risk prostate cancer. The relative paucity of high-risk patients prevented the authors from extending a similar recommendation to this subset.

DISCUSSION

Initial results and follow-up duration

The use of SBRT for prostate cancer has received considerable attention in recent years and multiple studies have demonstrated short-term outcomes comparable to established therapies. Currently, 8.8% of low-risk patients treated with RT at academic centers are receiving SBRT[29]. Advantages include the potential for

Table 1 Summary of stereotactic body radiotherapy prostate trials and retrospective analyses

Study	No. of patients	Dose	Median follow-up	Biochemical RFS	Overall survival	PSA response	BF[1]	PSA bounce
Madsen (*IJROBP*, 2007)	40	6.7 Gy × 5 Fx	41 mo	90% at 48 mo		18 mo time to nadir	3	"Few"
Pham (*IJROBP*, 2010)	40	6.7 Gy × 5 Fx	60 mo	93% at 60 mo	75% at 60 mo	Median nadir of 0.65 ng/mL at median time of 24 mo		22.50%
Boike (*JCO*, 2011)	15/15/15 (45 tot)	9/9.5/10 Gy × 5 Fx	30/18/12 mo	100% at median follow-up	100% at median follow-up	Mean < 0.4 ng/mL at 12 mo for all cohorts	0	"Multiple"
Katz (*BMC Urol*, 2010)	50/254 (304 tot)	7/7.25 Gy × 5 Fx	30/17 mo	35 Gy: 88% < 1 ng/mL PSA at 30 mo 36.25 Gy: 81% < 1 ng/mL PSA at 24 mo[2]	94%/99% at median follow-up. No deaths due to prostate cancer	28.1% < 0.5 ng/mL at 12 mo	4	37
Jabarri (*IJROBP*, 2012)	20/18 (38 tot)	9.5 Gy × 4/2 Fx	18.1/23.5 mo	100% at median follow-up		Median of 0.35 ng/mL at 18.3 mo	0	
King (*IJROBP*, 2012)	67	7.25 Gy × 5 Fx	2.7 yr	94% at 4 yr		Median of 0.50 ng/mL at follow-up	2	
McBride (*Cancer*, 2012)	34/10/1 (45 tot)	7.5/7.25 Gy × 5 Fx, 1 received "other regimen"	44.5 mo	95.5%/97.5% at 3 yr	97.7% at 3 yr	Median of 0.2 ng/mL at follow-up	0	9
Anwar (*Rad Oncol*, 2016)	24/26 (50 tot)	9.5/10.5 Gy boost in 2 Fx	42.7 mo	95%/95%/90% at 3/4/5 yr		Median nadir of 0.05 ng/mL at median time of 26.2 mo	4	2
Mantz (*Fontiers Rad Oncol*, 2014)	102	8 Gy × 5 Fx	Min. of 5 yr	99% at 6 yr		Mean of 0.27 ng/mL at 24 mo	1	15
Hannan (*Eur J Cancer*, 2016)	92	9/9.5/10 Gy × 5 Fx	54 mo (pooled phase Ⅰ/Ⅱ)	98.6% at 5 yr	94%/89.7% at 3/5 yr	Median of 0.125 ng/mL at 42 mo	1	19
Freeman (*Rad Oncol*, 2011)	41	7-7.25 Gy × 5 Fx	5 yr	93% at 5 yr		Median nadir of 0.3 ng/mL at follow-up	3	
Davis (*Cureus*, 2015)	437	7-7.4 Gy × 5 Fx, 9.5 Gy × 4 Fx, 19.5-29 Gy boost	20 mo	96.1% combined at 2 yr 99.0%/94.5%/89.8% for low/intermediate/high-risk at 2 yr		Median of 0.4 ng/mL at 24 mo	15	35

[1]Biochemical failure; [2]Values reflect only patients who did not receive hormone therapy.

improved therapeutic control and a reduced number of patient visits. However, the lack of long-term toxicity data combined with a relatively small number of patients enrolled in prospective trials prevents SBRT from superseding conventionally fractionated RT at the present time. Clinical results, discussed above and compiled in Tables 1-3, have demonstrated consistently favorable outcomes over the short-term using a variety of SBRT fractionation schedules for definitive and boost treatment. Overall, five fractions of 6.7 to 10.5 Gy per fraction were utilized. With range of follow-up varying from 18 to 60 mo, biochemical recurrence free survival was excellent, as later trials reported rates of greater than 93%.

Conventionally fractionated RT often requires long courses of treatment consisting of eight or more weeks of daily visits. The accelerated schedule that SBRT offers improves the logistic feasibility of treatment. While these initial SBRT reports are encouraging, longer follow up will be required to confirm that bRFS and an acceptably low rate of late toxicity can be maintained over the long term. Most current studies have yet to report data beyond five years and thus are not sufficient to allow for an unequivocal endorsement of SBRT in the treatment of prostate cancer.

Toxicity and dose per fraction

While continued follow-up and additional large-scale prospective studies are needed, certain conclusions can be inferred from the body of existing literature. Firstly, increasing per fraction dose beyond approximately 8 Gy appears to worsen toxicity without offering significantly improved progression-free survival. High-grade toxicity has not been reported in studies with doses between 7 and 8 Gy. Beyond 8 Gy per fraction, reports of both low- and high-grade toxicities increase measurably. Though rectal toxicity and early urinary toxicity are comparable to those seen with conventional fractionation, late urinary toxicity remains a concern[27,30]. The majority of studies evaluated here reported at least one instance of late grade 3 or higher urinary toxicity, often requiring instrumentation or transurethral resection of the prostate (TURP). This flare phenomenon has been found to peak between 12 and 18 mo post-treatment, though symptoms resolve by 24 mo in a majority of cases[31]. However, this trend remains a concern and should be further elucidated prior to large-scale adoption of SBRT

Table 2 Summary of genitourinary toxicities for included stereotactic body radiotherapy trials

Study	Acute			Late			Clinical notes
	Gr. 1	Gr. 2	Gr. ⩾ 3	Gr. 1	Gr. 2	Gr. ⩾ 3	
Madsen (*IJROBP*, 2007)	28%	21.50%	1 tot	25%	20%	0%	Gr. 3 event was urinary obstruction that resolved
Pham (*IJROBP*, 2010)				22.50%	12.50%	2.50%	All toxicities resolved
Boike (*JCO*, 2011)[1]	28.80%	22.20%	0%	13.30%	8.80%	2 tot	Gr. 3 events due to dysuria and cystitis
Katz (*BMC Urol*, 2010)[1]	74.60%	4.60%	0%	4.70%	5.10%	1 tot	
Jabarri (*IJROBP*, 2012)	29%	42%	0%	1 tot	8%	2 tot	One case each of urge incontinence and irritation requiring catheterization
King (*IJROBP*, 2012)[1]	Not reported	Not reported	Not reported	22.80%	5.30%	2 tot	Gr. 3 patients both underwent repeated urologic instrumentation for post-SBRT dysuria
McBride (*Cancer*, 2012)	59%	19%	0%	17%	17%	1 tot	Gr. 3 event was urinary obstruction requiring TURP
Anwar (*Rad Oncol*, 2016)	48%	37%	0%	21%	25%	1 tot	Gr. 3 event was urinary obstruction
Mantz (*Fontiers Rad Oncol*, 2014)	32.3% frequency, 16.6% dysuria, 7.8% retention		2 tot	19.6% frequency, 2.9% dysuria, 4.9% retention		0%	Gr. 3 events were urinary frequency
Hannan (*Eur J Cancer*, 2016)[1]	48.40%	22.00%	0%	24.20%	20.90%	5.50%	1 late Gr. 4 event (cystitis requiring ureteroileal diversion)
Freeman (*Rad Oncol*, 2011)	Not reported	Not reported	Not reported	25%	7%	1 tot	Gr. 3 event after repeated urologic instrumentation
Davis (*Cureus*, 2015)	19%/3%/3%[2]	2%/1%/1%[2]	0%	25%/4%/5%[2]	8%/2%/2%[2]	0%	

[1]Aggregate values for all cohorts; [2]Notation as follows: urinary frequency/urinary retention/cystitis. TURP: Transurethral resection of the prostate.

for low- and intermediate-risk prostate cancer.

Relatively few studies attempt to rigorously evaluate the impact of prostate volume on outcomes, and the overall conclusions are equivocal. A subset includes maximum volume cutoffs in the exclusion criteria, while others simply note the range of organ volumes among those enrolled. Chen *et al*[30] prospectively collected quality of life data for 204 prostate cancer patients treated with SBRT, with median follow up time of 3.9 years. Patients were treated to a dose of 35-36.25 Gy in 5 fractions. At 3 years post SBRT, EPIC-UI (Urinary Incontinence) score declined significantly; however, this was of borderline clinical significance. Notably, prostate volume was associated with UI score. Similarly, a second study evaluated 515 patients treated with SBRT to a dose of 35-36.25 Gy in 5 fractions. Of 336 patients with available prostate volumes, there was a higher incidence of grade 2 and 3 urinary toxicity with prostate volumes greater than 60 cc that trended towards statistical significance[19]. Conversely, a third study evaluated 216 patients treated with 35-36.25 Gy in 5 fractions, and found no correlation between urinary symptoms and prostate volume at the 2 year mark[31]. It is important to note that the mean prostate volumes for the first two studies were 39 cc and 65.3 cc, respectively; median prostate volume for the third study was 38 cc. For men with prostate volumes greater than 50 cc, Janowski *et al*[32] conducted a retrospective review of 57 patients with a median prostate volume of 62.9 cc (range 50-138.7cc). All patients were treated to 35-36.25 Gy in 5 fractions, and followed for a median of 2.9 years. The rate of grade 3 urinary toxicities was low, occurring in two patients. As there is limited data regarding toxicity with SBRT in the setting of larger volume prostates (> 100 cc), caution should be used when treating these patients.

Similar to large prostate volume, prior TURP may predict for worse toxicity, although large-scale data are unavailable. Bolzicco *et al*[21] prospectively accrued 100 patients for treatment with SBRT, to a dose of 35 Gy in 5 fractions. Of seven patients with prior TURP, three had late urinary toxicities (1% Grade 1, 1% Grade 2, 1% Grade 3). Also of note, there was only one patient with Grade 3 late urinary toxicity, and this patient had undergone urologic tests including cystoscopy and urethral dilatation. Similarly, Chen *et al*[33] report a single case of Grade 3 late urinary toxicity, in a patient with a large prostate and two prior TURP procedures.

Real-time tracking of the prostate

The role of improved technology cannot be overstated. Though rigorous evaluations of prostate movement are limited, it is commonly accepted that translation of 5 mm or more during a single treatment session is likely[34]. Real-time tracking of the prostate has the potential to markedly improve dose delivery to tumor tissue and minimize the exposure of surrounding non-involved structures. The majority of studies presented here made use of either CyberKnife or Calypso, and while there are no marked differences in toxicity, the prevailing sentiment among authors strongly favors real-time tracking. Furthermore, catheterization during treatment simulation improves urethral contour accuracy and may be advisable.

Table 3 Summary of gastrointestinal toxicities for included stereotactic body radiotherapy prostate trials

Study	Acute			Late			Clinical notes
	Gr. 1	Gr. 2	Gr. ⩾ 3	Gr. 1	Gr. 2	Gr. ⩾ 3	
Madsen (*IJROBP*, 2007)	26%	13%	0%	30%	7.50%	0%	Gr. 2 events were proctitis
Pham (*IJROBP*, 2010)				22.50%	7.50%	0.00%	All toxicities resolved
Boike (*JCO*, 2011)[1]	33%	22.50%	0%	22.20%	2 tot	1 tot	Gr. 4 event due to rectal ulcer
Katz (*BMC Urol*, 2010)[1]	74.90%	3.60%	0%	5.10%	2.30%	0%	
Jabarri (*IJROBP*, 2012)	21%	11%	0%	2 tot	1 tot	0%	
King (*IJROBP*, 2012)[1]	Not reported	Not reported	Not reported	14.00%	1 tot	0%	
McBride (*Cancer*, 2012)	31%	7%	0%	7%	7%	2 tot	Gr. 3 events were proctitis requiring ablation
Anwar (*Rad Oncol*, 2016)	42%	10%	0%	12.50%	0%	0%	
Mantz (*Fontiers Rad Oncol*, 2014)	0%	0%	0%	3 tot	0%	0%	Toxicity was rectal bleeding
Hannan (*Eur J Cancer*, 2016)[1]	37.40%	20.90%	2 tot	25.30%	13.20%	6.60%	1 acute and 2 late Gr. 4 events (one rectal bleed)
Freeman (*Rad Oncol*, 2011)	Not reported	Not reported	Not reported	13%	1 tot	0%	
Davis (*Cureus*, 2015)	4%/1%/1%[2]	1%/0%/0%[2]	0%	4%/3%/3%[2]	0%	0%	

[1]Aggregate values for all cohorts; [2]Notation as follows: Diarrhea/constipation/proctitis.

Future directions

Ongoing clinical trials seek to address some of the concerns discussed above. The SMART trial, initiated in 2009, is a phase Ⅱ study for stage T1-T2c prostate cancer using Calypso for real-time tracking and IMRT plan reoptimization. Patients are treated to 37 Gy in five fractions with a primary endpoint of urinary and gastrointestinal toxicity at 3 years, placing the focus on late complications. Enrollment has closed, though no results have been published to date. RTOG 0938 is a phase Ⅱ trial comparing 36.25 Gy delivered in 5 fractions to 51.6 Gy delivered in 12 fractions. This work builds upon RTOG 0415, an equivalence study comparing 70 Gy in 28 to a conventionally fractionated course of 73.8 Gy in 41 fractions. RTOG 0938 includes patients with T1-2a disease and mandates the use of intrafraction motion tracking. The primary endpoint is QOL at 1 year post-treatment, assessed by EPIC score. Again, the importance of toxicity is highlighted.

To date, the field has emphasized the role of SBRT in treating early stage, low- to intermediate-risk disease. A subset of studies presented here included high-risk patients and reported favorable results. Katz *et al*[18] noted a 7-year bRFS of 68.5% for high-risk cases while Anwar *et al*[35] reported 81% bRFS at 5 years. Additionally, Oliai *et al* report a 3-year freedom from biochemical failure of 77.1% for their high-risk cohort. These results, among others, suggest that SBRT may offer improved biochemical control as compared with conventionally fractionated RT and should be explored further in this context.

CONCLUSION

Initial studies examining the use of SBRT in the treatment of prostate cancer have demonstrated impressive rates of biochemical recurrence-free survival and PSA response, while maintaining a relatively favorable acute toxicity profile. Doses of 8 Gy or less per fraction have lower reported rates of toxicity with similar biochemical control rates compared to higher doses per fraction. Though we are cautiously optimistic that SBRT has the potential to serve as an alternative to conventionally fractionated RT in the treatment of prostate cancer, long-term follow-up is needed in order to evaluate whether biochemical control, overall survival, and late toxicity are maintained, or improved, as compared to the current standard of care.

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ORIGINAL ARTICLE

Basic Study

Characteristics of *Clostridium difficile* infection in patients hospitalized with myelodysplastic syndrome or acute myelogenous leukemia

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Author contributions: Shah K and Curtin BF collected data and wrote the manuscript; Chu C and Hwang D collected data; Flasar MH analyzed and interpreted the data; von Rosenvinge E supervised the study; all authors read and approved the final manuscript.

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Abstract

AIM

To evaluate factors associated with *Clostridium difficile* infection (CDI) and outcomes of CDI in the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) population.

METHODS

After IRB approval, all MDS/AML patients hospitalized at the University of Maryland Greenebaum Comprehensive Cancer Center between August 2011 and December 2013 were identified. Medical charts were reviewed for demographics, clinical information, development of CDI, complications of CDI, and mortality. Patients with CDI, defined as having a positive stool PCR done for clinical suspicion of CDI, were compared to those without CDI in order to identify predictors of disease. A *t*-test was used for comparison of continuous variables and chi-square or Fisher's exact tests were used for categorical

variables, as appropriate.

RESULTS

Two hundred and twenty-three patients (60.1% male, mean age 61.3 years, 13% MDS, 87% AML) had 594 unique hospitalizations during the study period. Thirty-four patients (15.2%) were diagnosed with CDI. Factors significantly associated with CDI included lower albumin at time of hospitalization ($P < 0.0001$), prior diagnosis of CDI ($P < 0.0001$), receipt of cytarabine-based chemotherapy ($P = 0.015$), total days of neutropenia ($P = 0.014$), and total days of hospitalization ($P = 0.005$). Gender ($P = 0.10$), age ($P = 0.77$), proton-pump inhibitor use ($P = 0.73$), receipt of antibiotics ($P = 0.66$), and receipt of DNA hypomethylating agent-based chemotherapy ($P = 0.92$) were not significantly associated with CDI.

CONCLUSION

CDI is common in the MDS/AML population. Factors significantly associated with CDI in this population include low albumin, prior CDI, use of cytarabine-based chemotherapy, and prolonged neutropenia. In this study, we have identified a subset of patients in which prophylaxis studies could be targeted.

Key words: *Clostridium difficile*; Acute myeloid leukemia; Cytarabine-based chemotherapy; Myelodysplastic syndrome; Neutropenia

Core tip: This study evaluates factors associated with the development and outcomes of *Clostridium difficile* infection (CDI) in patients with Myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML). Our findings demonstrate a high incidence of CDI with 15.2% of patients diagnosed with CDI during the 28-mo study period. Risk factors associated with the development of CDI include low albumin, prior history of CDI, chemotherapy within 30 d of hospitalization, cytarabine-based chemotherapy within 30 d of hospitalization, and increased duration of neutropenia and hospitalization.

Shah K, Curtin BF, Chu C, Hwang D, Flasar MH, von Rosenvinge E. Characteristics of *Clostridium difficile* infection in patients hospitalized with myelodysplastic syndrome or acute myelogenous leukemia. *World J Clin Oncol* 2017; 8(5): 398-404 Available from: URL: http://www.wjgnet.com/2218-4333/full/v8/i5/398.htm DOI: http://dx.doi.org/10.5306/wjco.v8.i5.398

INTRODUCTION

Clostridium difficile is a gram-positive, spore-forming, anaerobic bacterium that is the major cause of nosocomial diarrhea in the developed world. Over the last two decades the rate, morbidity, mortality, and costs of *C. difficile* infection (CDI) have risen dramatically[1]. Data from the United States Centers for Disease Control and Prevention show that the discharge diagnosis rate of CDI doubled from the 1990's into the 2000's[2,3]. CDI rates have increased considerably since that time, with a current estimate of almost half a million cases and 29000 deaths per year occurring in the United States alone[1]. This increase has not only been observed in hospitalized, elderly, and immunocompromised patients, but also in younger adults without significant comorbidities[4]. Patients who develop CDI have significant increases to their length of hospitalization[5]. According to a recent systematic review, attributable mean CDI costs range from $8911 to $30049 for hospitalized patients[6]. The sheer burden of CDI necessitates a search for more effective means of preventing and combating this infection.

Current statistics indicate that approximately 53000 new cases of leukemia will be diagnosed in the United States this year, 20000 of which will be acute myeloid leukemia (AML)[7]. Patients receiving treatment for myelodysplastic syndrome (MDS) or AML are at increased risk for developing CDI given their frequent neutropenic episodes, as well as exposure to antibiotics and chemotherapy[8]. Antineoplastic agents have antimicrobial properties, and numerous chemotherapeutic drugs have been associated with the development of CDI, including cisplatin, etoposide, bleomycin, paclitaxel, vinblastine, 5-fluorouracil, cyclophosphamide, methotrexate, doxorubicin, and cytarabine-based regimens[8]. Several risk factors for CDI in leukemia patients have been recently identified, which include receipt of chemotherapy, age > 65 years, admission at a teaching hospital, increased length of stay, diagnosis of acute rather than chronic leukemia, sepsis, and neutropenia[9,10].

The aim of this study is to evaluate factors associated with CDI and outcomes of CDI in the MDS and AML population. Outcomes of interest include mortality and severe morbidity such as Intensive Care Unit (ICU) admission, need for surgical intervention, or recurrence of CDI.

MATERIALS AND METHODS

The Institutional Review Board of the University of Maryland, Baltimore, approved this study and waived the requirement for informed consent (IRB# HP-00058296). All patients with a diagnosis of MDS or AML were identified through an electronic medical record database utilized by the University of Maryland Medical Center (UMMC). Inclusion criteria were: Age greater than or equal to 18 years, a diagnosis of MDS or AML, and hospitalization at the UMMC Greenebaum Comprehensive Cancer Center between August 2011 and December 2013. Charts were reviewed for demographics, clinical information, development of CDI, complications of CDI, and mortality. The starting point of data collection was identified as August 2011, when UMMC began to utilize the illumigene® *C. difficile* DNA amplification assay (Meridian Bioscience, Inc.). The assay uses loop-

Table 1 Characteristics of Patients with *C. difficile* infection *n* (%)

Variable (per patient)	CDI (*n* = 34)
Diagnosis	
AML	31 (91)
MDS	3 (9)
Gender	
Male	15 (44.1)
Female	19 (55.9)
PPI therapy[1]	22 (64.7)
Prior history of CDI	5 (14.8)
Receipt of chemotherapy[2]	31 (91)
Type of chemotherapy	
Cytarabine-based chemotherapy	21 (61.7)
DNA hypomethylating agent-based chemotherapy	11 (32.3)
Death/referral to hospice	8 (23.5)
Severity of CDI[3]	
Mild-moderate	29 (85.2)
Severe	4 (11.7)
Severe-complicated	1 (2.9)
Total number of CDI episodes during hospitalization	
1	31 (91)
2	3 (9)
Recurrence of CDI	4 (11.7)
ICU admission	8 (23.5)
Bowel perforation	0
Need for surgical intervention	1 (3)

[1]PPI therapy defined as use of PPI documented at the time of hospital admission; [2]Receipt of chemotherapy defined as being given within 30 d of hospital admission; [3]Severity as defined by the SHEA/IDSA Guidelines[12]. CDI: Clostridium difficile infection; AML: Acute myeloid leukemia; MDS: Myelodysplastic syndrome; PPI: Proton-pump inhibitor; ICU: Intensive care unit.

mediated isothermal DNA amplification to detect the tcdA 5′ region present in all toxigenic *C. difficile,* and has a sensitivity and specificity of 95.2% and 95.3%, respectively[11]. Our facility currently does not implement a two-step detection method for CDI.

Demographics and clinical data were recorded per patient encounter and included: Documented diagnosis of MDS or AML, age at diagnosis, gender, proton pump inhibitor (PPI) use during hospitalization or the within 30 d prior to hospitalization, any prior documented history of CDI, type of chemotherapy received during hospitalization or within 30 d prior to hospitalization, antibiotic use during hospitalization or within 30 d prior to hospitalization, total length of stay in days, albumin level at admission, duration of neutropenia during hospitalization, current episode of CDI as a recurrence, and documentation of death or referral to hospice. Data collected included factors previously associated with CDI and focused on investigating the primary aim of our study as described above. CDI was defined as a positive stool *C. difficile* test done in the setting of diarrhea, defined as the passage of 3 or more unformed stools in 24 or fewer consecutive hours[12]. Our laboratory policy does not permit *C. difficile* testing on formed stool, thus we are reasonably confident all patients had diarrhea. Recurrence of CDI was defined as CDI in the setting of a positive *C. difficile* stool assay as well as receipt of CDI treatment in the 8 wk prior to the current episode. Severity of CDI was determined based on the criteria set forth by the Society for Healthcare Epidemiology of America (SHEA) and Infectious Diseases Society of America (IDSA) guidelines[12]. Chemotherapeutic regimens were defined as cytarabine-based, DNA hypomethylating agent-based, or other regimens. Neutropenia was defined as an absolute neutrophil count of 500 cells/μL or less.

MDS and AML patients with CDI were compared to those patients that were not diagnosed with CDI in order to identify factors related to disease. A *t*-test was used for comparison of continuous variables and χ^2 or Fisher's exact tests were used for categorical variables, as appropriate (SAS, version 9.2). Statistical significance was defined as $P < 0.05$. As some patients were hospitalized multiple times, data analysis was performed on variables per hospital encounter. Total days of neutropenia as well as total days of hospitalization during the study period were analyzed per patient. A biomedical statistician performed the statistical review.

RESULTS

We identified 223 patients with MDS or AML that had 594 unique hospitalizations between August 2011 and December 2013. Sixty point one percent of the patients were male, the mean age was 61.3 years, 87% had AML, and 13% had MDS. Thirty-four of the patients (15.2%) were diagnosed with CDI during the study period. Of these, 44% were male, the mean age was 59.2 years, 91% had AML, 9% had MDS, and 35% were on a PPI at time of admission. Sixty point seven percent received cytarabine-based chemotherapy, and 32.3% received DNA hypomethylating agent-based chemotherapy. None of the patients with MDS who developed CDI received cytarabine-based chemotherapy. Eighty-five percent received antibiotics during hospitalization or within the 30 d prior to hospitalization. Twelve percent had recurrent CDI, eight required intensive care unit admission, and one underwent colectomy for CDI. According to the classification criteria set forth by the SHEA/IDSA guidelines, 85.2% had mild-moderate disease, 11.7% had severe disease, and 2.9% had severe-complicated disease[12]. Twenty-three point five percent of these patients died or were referred to hospice (Table 1).

Several factors were significantly associated with CDI when analyzed by hospital encounter (Table 2), including a lower albumin at the time of hospitalization (mean 2.8 g/dL in the CDI group *vs* 3.5 g/dL in the non-CDI group, $P < 0.0001$), prior history of CDI ($P < 0.0001$), receipt of any chemotherapy in within 30 d of hospitalization (92.1% in the CDI group *vs* 78.8% in the non-CDI group, $P = 0.048$), and receipt of cytarabine-based chemotherapy within 30 d of hospitalization (63.4% in the CDI group *vs* 45.5% in the non-CDI group, $P = 0.015$).

As some factors did not lend themselves to a per-hospital encounter analysis, we performed a per patient

Table 2 Comparison of myelodysplastic syndrome and acute myeloid leukemia patients with and without *Clostridium difficile* infection *n* (%)

Variable	No CDI (*n* = 556)	CDI (*n* = 38)	Significance (*P* value)	No CDI (*n* = 189)	CDI (*n* = 34)	Significance (*P* value)
Per encounter analysis						
Age on admission, mean (95%CI)	58.4 (57.0-59.8)	59.2 (54.6-63.9)	0.77			
Albumin level (g/dL) on admission, mean (95%CI)	3.5 (3.4-3.5)	2.8 (2.6-3.1)	< 0.0001			
AML (*vs* MDS) diagnosis	506 (91.0)	35 (92.1)	0.82			
Male gender	338 (60.8)	18 (47.4)	0.1			
Female gender	218 (39.2)	20 (52.63)	0.1			
Use of PPI therapy[1]	206 (37.0)	13 (34.21)	0.73			
Prior history of CDI	15 (2.7)	10 (23.32)	< 0.0001			
Antibiotic use	465 (84)	31 (83)	0.66			
Any chemotherapy[2]	438 (78.8)	35 (92.1)	0.048			
Cytarabine-based chemotherapy[2]	253 (45.5)	25 (65.79)	0.015			
DNA hypomethylating agent-based chemotherapy[2] (*n*)	165 (29.7)	11 (29.0)	0.92			
Other chemotherapy[2]	25 (4.5)	0 (0)	0.18			
Death or referral to hospice	82 (14.8)	8 (21.05)	0.29			
Per patient analysis						
Total days of neutropenia during study period				13.7 (11.3-16.0)	21.6 (14.5-28.7)	0.014
Total days of hospitalization during study period				22.7 (19.9-25.5)	40.8 (28.9-52.7)	< 0.0001

[1]PPI therapy defined as use of PPI documented at the time of hospital admission; [2]Receipt of chemotherapy defined as being given within 30 d of hospital admission. CDI: *Clostridium difficile* infection; MDS: Myelodysplastic syndrome.

analysis (Table 2) for total days of neutropenia during the study period (mean 21.6 d in the CDI group *vs* 13.7 d in the non-CDI group, $P = 0.014$), and total days of hospitalization during the study period (mean 40.8 d in the CDI group *vs* 22.7 d in the non-CDI group, $P = 0.005$).

DISCUSSION

Our findings demonstrate a high incidence of CDI in our MDS and AML population with 15.2% of patients diagnosed with CDI during the 28-mo study period. This is comparable to previous reports. In a retrospective study of AML patients receiving chemotherapy, the incidence of CDI was 18%[13]. In another similar study, the incidence was 12%[14]. Within this overall high-risk group, we identified several factors associated with CDI. Specifically, CDI is significantly associated with low albumin level at time of hospitalization, prior history of CDI, receipt of any chemotherapy within 30 d of hospitalization, receipt of cytarabine-based chemotherapy within 30 d of hospitalization, total length of neutropenia and total length of hospitalization.

Similar to previously published findings, we found a higher rate of CDI in women, though this result was not statistically significant ($P = 0.10$)[1]. While other studies have identified associations between age and PPI use and risk for CDI[13], age ($P = 0.77$) and PPI use ($P = 0.73$) were not associated with CDI in our population. In addition, use of DNA hypomethylating agent-based chemotherapy ($P = 0.92$) was not associated with CDI. No differences in mortality or referral to hospice rates during the study period were identified between CDI and non-CDI groups ($P = 0.29$). It is well established that the greater the antibiotic exposure, the greater the risk of CDI[12,13]. However, infections during a neutropenic state are associated with high mortality rates, and thus antibiotic prophylaxis is indicated in patients with high-risk neutropenia per American Society of Clinical Oncology guidelines[15]. Fluoroquinolones are generally the agents of choice in these situations. The emergence of the NAP1/BI/027 hypervirulent strain is associated with an increased incidence of CDI over the past 15 years[16]. Fluoroquinolone resistance characterizes the NAP1/BI/027 strain[16], which may be one reason for increased risk of CDI in this population. In our study, 85% of patients received antibiotic therapy. Interestingly, antibiotic usage was not a significantly associated with CDI ($P = 0.66$). This likely reflects insufficient power to detect a difference given the high rate of antibiotic use in this population. Previous studies examining strategies to improve antibiotic prescribing practices of providers have shown mixed results in the reduction of CDI incidence[17]. However, reducing the duration and potency of antibiotics used, particularly after initial presentation of CDI, would be an interesting area of study for the MDS and AML population.

Consistent with our findings, low albumin levels have previously been established as a risk factor for the development of CDI[18]. A low albumin level may indicate poor baseline health status, malnutrition, or the presence of other comorbidities such as cirrhosis or nephrotic syndrome, all which may increase susceptibility to CDI[19,20]. Also, low albumin may be found in cases of diarrhea and loss of protein due to mucositis/enterocolitis in patients after chemotherapy for AML. A prior history of CDI was associated with CDI, as demonstrated in previous studies[21]. Prior history of CDI may predispose a patient to future episodes of CDI due to patient colonization, environmental contamination, or the presence of persistent risk factors.

The majority of patients in our study had a confir-

med diagnosis of AML and received treatment with cytarabine-based chemotherapy. Based on our findings, any chemotherapy, and cytarabine-based therapy in particular, was associated with development of CDI. This may be related to neutropenia, as total days of neutropenia was also significantly increased in patients that developed CDI. Chemotherapeutics are also known to disrupt enteric bacterial populations and the resulting dysbiosis may predispose to CDI[8]. While there is a paucity of data on the effect of different chemotherapeutic regimens on the gastrointestinal microbiome, cytotoxic changes may create a favorable environment for the proliferation of *C. difficile*. Microbial data suggests that chemotherapeutics may select for colonization of *C. difficile* and *Enterococcus faecium*[22].

We evaluated several factors that did not prove to be significantly associated with CDI in our MDS and AML population, including age, gender, PPI use, use of DNA hypomethylating agent-based chemotherapy, and antibiotic use. In theory, PPI therapy may increase the risk of CDI by increasing the ability of *C. difficile* spores to survive in the lumen of the gastrointestinal tract. While there has been controversy regarding their significance, a meta-analysis demonstrated a significant association between PPI use and risk of developing CDI (OR = 1.74, 95%CI: 1.47-2.85)[23]. Within the same study there appears to be increased risk with concomitant use of antibiotics and PPIs, and increased risk of recurrence with PPI use[23]. Non-cytarabine based chemotherapy, which in the case of our study was primarily DNA-hypomethylating agents, was not associated with CDI. We hypothesize that cytarabine-based agents are generally more caustic and induce a greater period of neutropenia, thus providing a more favorable environment for CDI in comparison to less cytotoxic agents.

We believe that our findings will inform future CDI prophylaxis studies in the high-risk MDS and AML population. We have identified a subset of this population, namely those with low albumin, prior CDI, or receipt of cytarabine-based chemotherapy, who can be identified at time of hospital admission as being especially high-risk for CDI. Recently, metronidazole prophylaxis has been proposed as a possible strategy for CDI prevention, however data specifically looking at patients with malignancies has not been supportive of prophylactic antibiotic treatment to prevent CDI[24,25]. In addition, the anti-toxin monoclonal antibody bezlotoxumab was recently approved by the FDA, and a toxoid vaccine in phase Ⅲ clinical study is likely to be available soon[26,27]. Studies of these agents for CDI prophylaxis in our high-risk patient population are warranted.

Our study is not without limitations. Our study is retrospective and took place in a single tertiary medical center. We included primarily AML patients with a high degree of medical complexity, and our findings may not be generalizable to other populations. Additionally, many of the patients had prolonged hospital stays or numerous admissions throughout the testing period, and our study design was ill-equipped to evaluate temporal relationships between chemotherapy and CDI onset. Another limitation is our inability to analyze the degree in which antibiotics predict development of CDI in this population. While antibiotic usage was not found to be associated with CDI in our study, the widespread use of antibiotics makes this difficult to assess. While antibiotic exposure is not necessary for the development of CDI, it is likely to contribute to our population's overall CDI risk[10].

In conclusion, CDI is common in our MDS/AML population. Factors significantly associated with CDI include low albumin, prior history of CDI, use of cytarabine-based chemotherapy, and prolonged neutropenia. Length of hospitalization is also associated with CDI; however, this is likely both a cause and effect of CDI. Prophylactic strategies to lower the burden of CDI in MDS/AML patients are needed. In this study, we have identified a subset of this high-risk population in which prophylaxis studies could be targeted. These findings are novel and increase our understanding of CDI in this patient population as well as open new frontiers of research.

COMMENTS

Background

Acute myelogenous leukemia (AML) and Myelodysplastic syndrome (MDS) are blood borne malignancies that require strong treatments with heavy doses of chemotherapy, which leaves these patient's susceptible to opportunistic infections. *Clostridium difficile* infection (CDI) remains a major cause of nosocomial diarrhea and is of significant importance to the immunosuppressed population, such as those receiving chemotherapies for AML and MDS.

Research frontiers

CDI has been recognized as a major contributor of increased morbidity and mortality in hospitalized patients. New treatment regimens, such as vaccinations, immunotherapy, and fecal transplantation are currently undergoing evaluation. It is essential to identify certain susceptible populations in which targeted therapy for CDI can be investigated. Patients with AML and MDS are particularly susceptible to CDI and further characterization of CDI in this population is warranted.

Innovations and breakthroughs

The authors have found that CDI is common in this specific patient populations. Factors significantly associated with CDI in this population include low albumin, prior CDI, use of cytarabine-based chemotherapy, and prolonged neutropenia. The authors have identified a subset of patients in which prophylaxis studies could be targeted

Applications

By identifying and characterizing CDI within this specific patient population, the authors have identified a cohort of patients that would benefit from future novel CDI therapies and possible CDI prophylaxis. The authors have also identified risk factors that would enable providers to recognize patients that are particularly susceptible for identifying CDI and adjusting their management accordingly.

Terminology

CDI was defined as a positive stool *C. difficile* test done in the setting of diarrhea, defined as the passage of 3 or more unformed stools in 24 or fewer consecutive hours. Recurrence of CDI was defined as CDI in the setting of a positive *C. difficile* stool assay as well as receipt of CDI treatment in the 8 wk prior to the current episode. Severity of CDI was determined based on the

criteria set forth by the Society for Healthcare Epidemiology of America (SHEA) and Infectious Diseases Society of America guidelines. Chemotherapeutic regimens were defined as cytarabine-based, DNA hypomethylating agent-based, or other regimens. Neutropenia was defined as an absolute neutrophil count of 500 cells/μL or less.

Peer-review

The authors have shown that CDI is common in the MDS/AML population. Factors significantly associated with CDI in this population include low albumin, prior CDI, use of cytarabine-based chemotherapy, and prolonged neutropenia. The findings are worthy of sharing with the scientific community.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Factors influencing response to ingenol mebutate therapy for actinic keratosis of face and scalp

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Abstract

AIM

To determine factors independently influencing response to ingenol mebutate therapy and assess efficacy on clinical setting of non-hypertrophic non-hyperkeratotic actinic keratosis (AK).

METHODS

Consecutive patients affected by non-hypertrophic non-hyperkeratotic AKs of the face or scalp were enrolled to receive ingenol mebutate 0.015% gel on a selected skin area of 25 cm^2 for 3 consecutive days. Local skin reactions were calculated at each follow up visit using a validated composite score. Efficacy was evaluated by the comparison of clinical and dermoscopic pictures before the treatment and at day 57, and classified as complete, partial and poor response.

RESULTS

A number of 130 patients were enrolled, of which 101 (77.7%) were treated on the face, while 29 (22.3%) on the scalp. The great majority of our study population (n = 119, 91.5%) reached at least a 75% clearance of AKs and, in particular, 58 patients (44.6%) achieved a complete response while 61 (46.9%) a partial one.

Logistic backward multivariate analysis showed that facial localization, level of local skin reaction (LSR) at day 2, the highest LSR values and level of crusts at day 8 were factors independently associated with the achievement of a complete response.

CONCLUSION

Ingenol mebutate 0.015% gel, when properly applied, is more effective on the face than on the scalp and efficacy is directly associated to LSR score.

Key words: Ingenol mebutate; Actinic keratosis; Facial and scalp lesions; Skin reactions; Dermoscopic feature

Core tip: Ingenol mebutate 0.015% gel is an effective treatment for non-hypertrophic non-hyperkeratotic actinic keratosis of face and scalp. Facial lesions are more prone to achieve a complete response to this therapy than those located on the scalp. Facial localization and the highest levels of local skin reaction, in particular the amount of crusting, are predictive for complete response to ingenol mebutate 0.015% gel therapy in a real clinical setting.

Skroza N, Proietti I, Bernardini N, Balduzzi V, Mambrin A, Marchesiello A, Tolino E, Zuber S, La Torre G, Potenza C. Factors influencing response to ingenol mebutate therapy for actinic keratosis of face and scalp. *World J Clin Oncol* 2017; 8(5): 405-411 Available from: URL: http://www.wjgnet.com/2218-4333/full/v8/i5/405.htm DOI: http://dx.doi.org/10.5306/wjco.v8.i5.405

INTRODUCTION

For a long time dermatologists have questioned if actinic keratosis (AK) should be considered as a precancerous lesion or an early squamous cell carcinoma (SCC). Apart from academic debate, it is actually clear that AKs have a low but definite potential to become invasive and even metastatic and that this risk increases over time[1].

Since it is impossible to predict which AK will progress to SCC and given the high prevalence of AKs in people with fair photo-types, chronically exposed to ultraviolet (UV) rays, treatment is recommended[2].

Conventional treatments for AK include cryotherapy, laser-therapy, surgical excision, photodynamic therapy, diclofenac 3% gel, imiquimod 5% and 5-fluorouracil creams[3,4].

Ingenol mebutate 0.015% gel, obtained by the sap of the plant Euphorbia peplus, has been recently approved in Europe for the treatment of non-hypertrophic non-hyperkeratotic AKs of face and scalp, which mainly correspond to Ⅰ and Ⅱ histopathologic categories[5,6].

The mechanism of action of ingenol mebutate has been partially explained with a rapid cytotoxic activity at higher concentration and with the activation of immune system at lower concentration[7]. The long-lasting immune surveillance and the clearance of single tumour cell clones within cancerization field, could justify the low recurrence rates of AKs observed after treatment[8].

To the best of our knowledge, no studies have assessed factors independently influencing the response to ingenol mebutate therapy. Efficacy data of phase Ⅲ trials have not been widely confirmed on a large real clinical setting to date[9-11].

These studies reported a higher efficacy of ingenol mebutate 0.015% gel in patients experiencing more severe local skin reactions (LSRs); however they didn't investigate how the single components of the composite LSR score could influence the response to treatment.

We conducted a prospective study to determine which factors, among age, gender, head site and LSR score, could independently predict the response to 0.015% ingenol mebutate treatment and to assess the efficacy of this therapy in a real clinical setting.

MATERIALS AND METHODS

Study population

We (GLV and RP) enrolled consecutive patients, aged ⩾ 18 years, affected by non-hypertrophic non-hyperkeratotic AKs of face and scalp, who were attending our outpatient clinic from April 2014 to March 2015.

The diagnosis of AK was performed both clinically and dermoscopically, respectively based on the presence of erythematous macular lesions with or without a slightly scaly surface, and on the identification of the typical red pseudonetwork, corresponding to grade Ⅰ AK, or strawberry pattern, corresponding to grade Ⅱ AK[12].

The presence of a skin cancer other than AK in the selected skin area was considered as an exclusion criteria. Furthermore, if at least one AK of the selected area had been treated by non-ablative methods within the previous year, patient was excluded from the study.

Treatment procedure

Ingenol mebutate 0.015% gel was applied by the same physician (GLV) for 3 consecutive days on a selected skin area of 25 cm^2, which included 4 to 8 AKs.

Each enrolled patient gave written informed consent for clinical and dermoscopic digital documentation and the ethical committee approval was waived.

Outcome assessment

Clinical and dermoscopic pictures were collected at baseline and at each control visit (day 2, 3, 8, 15, 29 and 57).

Local skin reactions (LSR) score was calculated at each control visit, using a validated composite score (ranging from 0 to 24) given by the sum of 6 single scores for erythema, flaking/scaling, crusting, swelling, vesiculation/pustulation and erosion/ulceration; with grade 0 representing no reaction while grade 4 indicating a skin reaction extending beyond the treated are[13].

Table 1 Demographic and response data of the whole study population *n* (%)

Factors		Value
Age (yr), mean ± SD		72.2 ± 10.3
Gender	M	91 (70)
	F	39 (30)
	Total	130
Head site	Face	101 (77.7)
	Scalp	29 (22.3)
	Total	130
Response	Poor	11 (8.5)
	Partial	61 (46.9)
	Complete	58 (44.6)
	Total	130

Efficacy was evaluated comparing clinical and dermoscopic pictures at baseline and at day 57 and response was classified as complete, partial (⩾ 75% clearance) or poor (< 75% clearance).

Statistical analysis

Statistical analyses were performed using the IBM SPSS 21.0 package (Statistical Package for Social Sciences, SPSS Inc., Chicago, Ill.).

Data is expressed as mean standard deviation. To analyse factors influencing efficacy of 0.015% ingenol mebutate therapy, we used Spearman's rho coefficient to assess significant correlations, which were subsequently quantified *via* univariate logistic regression. Furthermore, a logistic multivariate regression backward model was constructed to identify major independent factors that showed a significant difference ($P < 0.10$) on univariate analysis, that have an influence on complete response. The statistical significance was set at $P < 0.05$.

RESULTS

Study population and efficacy data

Demographic and efficacy data are listed in Table 1. A number of 130 patients were enrolled, 91 (70.0%) were males and 39 (30.0%) were females, with a mean age (standard deviation) of 72.2 (10.3) years. All the patients completed the 3 applications of ingenol mebutate 0.015% gel, as scheduled; the majority, 101 (77.7%) were treated on the face, while 29 (22.3%) on the scalp.

Regarding efficacy, the great majority of our study population (119, 91.5%) reached at least a 75% clearance of AKs, in particular 58 patients (44.6%) achieved a complete response and 61 (46.9%) a partial one; while poor responders were only 11 (8.5%).

Figure 1 shows the clinical and dermoscopic pictures of a patient treated on the scalp, before and after the therapy.

Local skin reaction data

Figure 2 and Table 2 report data about the "number of patients with positive scores" and "mean values" of LSR composite and single scores at each follow up visit.

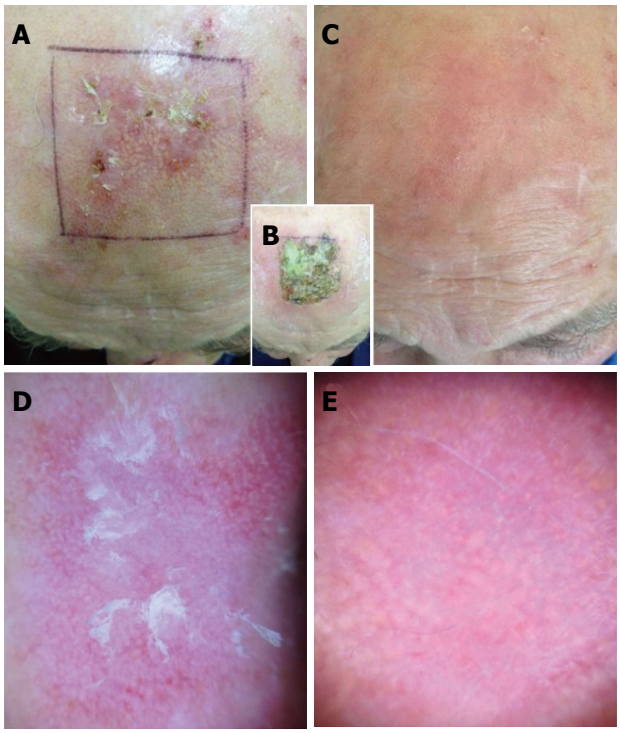

Figure 1 Patient treated with ingenol mebutate for actinic keratosis of the scalp. A and C: Clinical images of the treated area before and after (day 57) the therapy, respectively; B: Local skin reaction to ingenol mebutate at day 8 showing a grade 3 crusting reaction and erythema exceeding the treated area (grade 4); D: Dermoscopic image of an actinic keratosis of the treated area at baseline showing red pseudonetwork and scaling in the central area; E: Dermoscopic picture of the same skin area at day 57 showing the complete disappearance of the preexisting actinic keratosis.

Each patient enrolled experienced at least one LSR, but no one reported systemic symptoms.

The highest number of patients involved and the highest mean scores were reached at day 3 for both composite and all single scores, with the exception of crusting and flaking/scaling, reaching the highest level at day 8 and 15, respectively.

These 2 components were the less represented at day 2 [4 patients (3.1%) had flaking/scaling and only 2 (1.5%) had crusts, with mean values of 0.05 ± 0.28 and 0.02 ± 0.12, respectively] and totally disappeared in the whole population since day 57.

Erythema was the only LSR component involving the entire study population (at day 2 and 3) and the only, still present at day 57 in 49 patients (37.7%), with a mean score of 0.38 ± 0.50.

Swelling reached the highest levels at day 2 and 3 [114 (87.7%) and 125 (96.2%) patients, with 1.48 ± 0.82 and 2.38 ± 1.08 mean scores, respectively], but quickly reduced afterward, becoming totally absent since day 57.

Grade 4 swelling was observed in 23 (17.7%) patients and presented as periorbital edema following the application of ingenol mebutate gel on forehead and temporal areas; it resolved within day 15 in all cases.

Vesiculation/pustulation were the first signs to

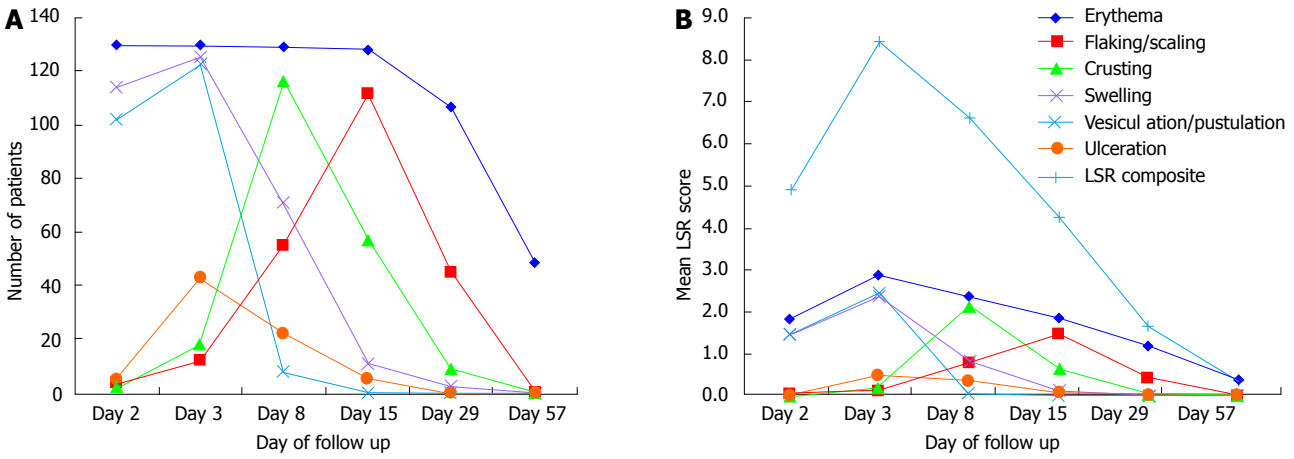

Figure 2 Number of skin reactions and scores at each follow up visit. A: The number and features of different skin reactions over the time; B: Mean values describing the severity of each skin reaction and the LSR composite score (light blue line). Skin reactions included: Erythema (blue), flaking/scaling (red), crusting (green), swelling (purple), vesiculation/pustulation (light blue), and ulceration (orange). LSR: Local skin reaction.

Table 2 Number of patients with positive scores and mean values of local skin reaction composite and single scores at each follow up visit

		Day 2	Day 3	Day 8	Day 15	Day 29	Day 57
Erythema	*n* (%)	130 (100)	130 (100)	129 (99.2)	128 (98.5)	107 (82.3)	49 (37.7)
	mean ± SD	1.82 ± 0.68	2.87 ± 0.58	2.38 ± 0.78	1.86 ± 0.81	1.19 ± 0.77	0.38 ± 0.50
Flaking/scaling	*n* (%)	4 (3.1)	12 (9.2)	55 (42.3)	112 (86.2)	45 (34.6)	0
	mean ± SD	0.05 ± 0.28	0.11 ± 0.39	0.78 ± 1.00	1.49 ± 0.87	0.43 ± 0.65	0
Crusting	*n* (%)	2 (1.5)	18 (13.8)	116 (89.2)	57 (43.8)	9 (6.9)	0
	mean ± SD	0.02 ± 0.12	0.17 ± 0.45	2.16 ± 0.98	0.65 ± 0.89	0.10 ± 0.39	0
Swelling	*n* (%)	114 (87.7)	125 (96.2)	71 (54.6)	11 (8.5)	2 (1.5)	0
	mean ± SD	1.48 ± 0.82	2.38 ± 1.08	0.85 ± 0.98	0.13 ± 0.55	0.02 ± 0.12	0
Vesiculation/pustulation	*n* (%)	102 (78.5)	123 (94.6)	8 (6.2)	0	0	0
	mean ± SD	1.48 ± 0.93	2.45 ± 0.86	0.06 ± 0.24	0	0	0
Ulceration	*n* (%)	5 (3.8)	43 (33.1)	22 (16.9)	5 (3.8)	0	0
	mean ± SD	0.04 ± 0.19	0.48 ± 0.74	0.36 ± 0.90	0.08 ± 0.45	0	0
LSR composite	mean ± SD	4.89 ± 2.14	8.43 ± 2.38	6.62 ± 2.44	4.25 ± 1.72	1.66 ± 1.28	0.35 ± 0.49

LSR: Local skin reaction.

disappear, being widely present at day 2 and 3 [102 (78.5%) and 123 (94.6%) patients, with 1.48 ± 0.93 and 2.45 ± 0.86 mean scores, respectively], but only observable in 8 patients (6.2%) at day 8 and completely absent since day 15.

Ulceration was the least observed LSR component, being present in a maximum of 43 patients (33.1%) at day 3 and early disappearing in the entire population since day 29.

Spearman's correlation

Spearman rho analysis highlighted significant correlations among response and gender, head site and the maximum level of the LSR composite score ($\rho = 0.189$, $P = 0.031$; $\rho = -0.258$, $P = 0.003$; $\rho = 0.449$, $P < 0.001$, respectively).

Furthermore, all the maximum levels of single scores, but flaking/scaling, resulted to be correlated to response (erythema: $\rho = 0.351$, $P < 0.001$; vesiculation: $\rho = 0.329$, $P < 0.001$; crusting: $\rho = 0.255$, $P = 0.003$; swelling: $\rho = 0.365$, $P < 0.001$; ulceration: $\rho = 0.194$, $P = 0.027$).

Regarding the single follow up visits, a significant correlation with response was reported for LSR composite score at day 2, 3, 8 and 15 ($\rho = 0.455$, $P < 0.001$; $\rho = 0.484$, $P < 0.001$; $\rho = 0.325$, $P < 0.001$; $\rho = 0.234$, $P = 0.007$, respectively), for erythema at every follow up visit (day 2: $\rho = 0.400$, $P < 0.001$; day 3: $\rho = 0.351$, $P < 0.001$; day 8: $\rho = 0.314$, $P < 0.001$; day 15: $\rho = 0.270$, $P = 0.002$; day 29: $\rho = 0.282$, $P = 0.001$; day 57: $\rho = 0.189$, $P = 0.032$), for crusting, swelling, vesiculation/pustulation and ulceration at days 3 ($\rho = 0.180$, $P = 0.041$; $\rho = 0.372$, $P < 0.001$; $\rho = 0.329$, $P < 0.001$; $\rho = 0.215$, $P = 0.014$, respectively) for swelling and vesiculation at day 2 ($\rho = 0.357$, $P < 0.001$; $\rho = 0.418$, $P < 0.001$, respectively) and for crusting and swelling at day 8 ($\rho = 0.288$, $P = 0.001$; $\rho = 0.237$, $P = 0.007$, respectively).

Univariate analysis

The univariate logistic regression analysis confirmed that the factors highlighted by Spearman's correlation were all good predictors of complete response to

Table 3 Univariate logistic regression analysis

			OR	95%CI for OR		*P* value
				Lower	Upper	
Gender			2.30	1.07	4.94	0.033[a]
Head site			4.07	1.53	10.83	0.005[a]
Max values	LSR composite		1.55	1.27	1.89	< 0.001[a]
		Erythema	3.90	1.86	8.19	< 0.001[a]
		Crusting	1.85	1.18	2.92	0.008[a]
		Swelling	2.24	1.50	3.35	< 0.001[a]
		Vesiculation/pustulation	2.76	1.55	4.94	0.001[a]
Day 2	LSR composite		1.70	1.36	2.12	< 0.001[a]
		Erythema	3.83	2.05	7.17	< 0.001[a]
		Vesiculation/pustulation	2.82	1.74	4.56	< 0.001[a]
		Swelling	2.73	1.64	4.55	< 0.001[a]
Day 3	LSR composite		1.65	1.34	2.05	< 0.001[a]
		Erythema	3.90	1.86	8.19	< 0.001[a]
		Vesiculation/pustulation	2.76	1.55	4.94	0.001[a]
		Swelling	2.22	1.51	3.28	< 0.001[a]
		Ulceration	1.72	1.06	2.79	0.028[a]
Day 8	LSR composite		1.25	1.07	1.47	0.006[a]
		Erythema	2.44	1.45	4.13	0.001[a]
		Swelling	1.55	1.06	2.25	0.022[a]
		Crusting	1.76	1.17	2.64	0.006[a]
Day 15	LSR composite		1.27	1.02	1.59	0.030[a]
		Erythema	1.93	1.22	3.05	0.005[a]
Day 29		Erythema	2.06	1.26	3.37	0.004[a]
Day 57		Erythema	2.03	1.01	4.09	0.047[a]

Factors predicting the response to ingenol mebutate 0.015% therapy. [a]$P < 0.05$. OR: Odds ratio; LSR: Local skin reaction.

ingenol mebutate 0.015% therapy, with the exclusion of crusting at day 3 and the highest values of ulceration (OR = 2.32, 95%CI: 0.99-5.46, $P = 0.053$ and OR = 1.35, 95%CI: 0.93-1.96, $P = 0.113$, respectively) (Table 3).

More specifically, females were 2 times more likely to risk facial lesions than males, and were almost 4 times more likely to achieve a complete response than scalp ones.

Concerning local skin reactions, both the maximum levels and the values at day 2, 3, 8 and 15 of the composite score were associated with increased odds to achieve a complete response, ranging from 1.27 to 1.70.

Similarly, for erythema, both the maximum values and the levels at each follow up visit were associated with a complete response.

The maximum levels of crusting, swelling and vesiculation/pustulation gave also an increased odd to achieve a complete response, as well as the scores of swelling and vesiculation/pustulation at day 2 and 3 and of swelling and crusting at day 8.

Finally, ulceration at day 3 was also predictive of complete response to therapy.

Multivariate analysis

Multivariate backward logistic regression analysis showed that patients with facial lesions were almost 5 times more likely to achieve a complete response than those treated on the scalp (OR = 5.19, 95%CI: 1.51-17.86, $P = 0.009$); LSR composite score at day 2 resulted as a predictive factor of complete response, with 14.6% higher odds for each point of score added (OR = 1.46, 95%CI: 1.08-1.97, $P = 0.014$). Furthermore, also the maximum level of LSR composite score was associated with complete response to ingenol mebutate therapy, but with a lower statistical significance (OR = 1.50, 95%CI: 1.02-2.21, $P = 0.038$). Finally, regarding single scores, we found that patients with higher crusting reactions at day 8 were more likely to achieve a complete response, with 19.4% higher odds for each point of score added (OR = 1.94, 95%CI: 1.18-3.20, $P = 0.009$) (Table 4).

DISCUSSION

Ingenol mebutate gel was recently introduced as a safe and effective therapeutic option for non-hypertrophic non-hyperkeratotic AK at the dosage of 0.015% for face and scalp[14,15].

Phase Ⅲ trials reported complete clearance rates of 42.2% and partial response rates of 63.9%, for the treatment of facial and scalp AKs with ingenol mebutate, 5 however less is known about the factors influencing the response to treatment[16].

In the present study, we achieved complete and partial responses in 44.6% and 46.9% of cases, respectively; furthermore, ingenol mebutate 0.015% gel therapy resulted to be independently related to both the head site and the level of LSR, with a higher efficacy on facial lesions, compared to scalp ones and in case of more severe LSRs. Level of crusting at day 8 was independently associated with the achievement of a complete response.

Table 4 Multivariate logistic regression backward analysis[1]

	OR	95%CI for OR		*P* value
		Lower	Upper	
Head site	5.19	1.51	17.86	0.009[a]
LSR composite day 2	1.46	1.08	1.97	0.014[a]
Crusting day 8	1.94	1.18	3.20	0.009[a]
LSR composite max	1.50	1.02	2.21	0.038[a]

[1]Factors predicting response to ingenol mebutate 0.015% therapy. Logistic backward multivariate regression model. Reported OR mutually adjusted for all variables in the model. Variables in the model: Gender: Male (M), female (F); head site: Scalp, face; erythema at day 2, 3, 8, 15, 29, 57 and max; crusting at day 8 and max; swelling at day 2, 3, 8 and max; vesiculation/pustulation at day 2, 3 and max; ulceration at day 3; LSR composite at day 2, 3, 8, 15 and max. [a]$P < 0.05$. OR: Odds ratio; LSR: Local skin reaction.

Previous studies showed a greater efficacy of ingenol mebutate on AKs located on the face compared to scalp lesions, but the reason has not been clarified so far. In our opinion a possible explanation could be related to the lower rate of self-application errors on face than on scalp; however, in the present study, we obtained the same results even performing a physician-assisted application[17]. Therefore, other factors should be investigated to explain these findings, such as local differences in skin architecture, microbiota and ph.

Regarding the LSR composite score, we observed that both the highest levels and the values at day 2 were independently associated to complete response. The vast majority of our study population reached the highest values of LSR composite score at day 2.

The weight of each component of the composite score at each follow up visit was further evaluated and related to drug efficacy.

Erythema was the only component present at each evaluation and it was closely associated with response in univariate logistic regression analysis. Intriguingly, in multivariate analysis, when the weight of each variable was mutually adjusted for all variables in the model, erythema no longer could be associated with the response to therapy.

The highest levels of swelling and vesiculation/pustulation and the levels of these components reported in the first week after treatment were significantly associated to response in univariate analysis, but not in the multivariate model.

Conversely, the level of crusting at day 8 was the only single component of LSR composite score independently associated with the achievement of a complete response to ingenol mebutate therapy. A possible explanation of this finding could be related to the fact that the other parameters, in particular swelling and vesiculation/pustulation, probably reached their peak between day 3 and 8 follow up visits, so we couldn't register the highest levels of these reactions. This is also supported by the fact that crusts are strictly related to the occurrence of vesicles and pustules, resulting from the drying of their fluid content.

Differently from phase Ⅲ trials in which the first follow up was set at day 4, we evaluated LSRs at day 2 and 3, during physician-assisted application of ingenol.

Physician assisted application seems to be very effective in limiting withdrawal due to LSRs therefore improving adherence, in particular in elderly patients; however, a direct comparison with self-application was not performed.

Other limitations of the present study were the absence of long term efficacy, safety and cosmetic data, the absence of a quantitative evaluation of symptoms, such as pruritus, burn and pain and the low number of patients treated on the scalp, compared to the face group. However, facial localization demonstrated to be independently associated to complete response in multivariate analysis; whereas, this was not the case for patients treated on the scalp, due to the low number of patients that were treated. To obtain a more reliable result a test should be made on a higher number of patients.

On the basis of our findings we suggest that physician-assisted application of ingenol mebutate, at least for the first 2 d, could be very effective in order to improve adherence and patient satisfaction, maximize the results and minimize the risk of application errors. The severity of LSRs at day 2 and the level of crusting at day 8 should be considered as the best predictors of response to treatment.

In conclusion, our experience demonstrates that ingenol mebutate 0.015% gel is safe and effective when applied correctly. This treatment seems to be more effective on the face than on the scalp and the efficacy seems to be directly related to the level of LSR.

COMMENTS

Background

Actinic keratosis (AK) is considered an *in situ* squamous cell carcinoma, therefore treatment is mandatory.

Research frontiers

Ingenol mebutate 0.015% gel was recently approved for the treatment of non-hypertrophic non-hyperkeratotic AK of face and scalp.

Innovations and breakthroughs

This study considers severe local skin reaction (LSR) the most important factor influencing the response to ingenol mebutate therapy for actinic keratosis.

Applications

This study demonstrates that ingenol mebutate 0.015% gel is safe and effective when applied correctly. This treatment seems to be more effective on the face than on the scalp and the efficacy seems to be directly related to the level of LSR.

Peer-review

This is an interesting study regarding the use of ingenol mebutate therapy for actinic keratosis of face and scalp, and the factors which may affect the treatment response. The study was well-performed, the results are novel and interesting, and the findings should be clinically relevant and useful.

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ORIGINAL ARTICLE

Observational Study

Prophylactic lateral pelvic lymph node dissection in stage Ⅳ low rectal cancer

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Institutional review board statement: This study was performed in accordance with the Helsinki Declaration, and the Ethics Committee of the School of Medicine. Niigata University approved the study protocol (approval number: 2330).

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Abstract

AIM

To assess the clinical significance of prophylactic lateral pelvic lymph node dissection (LPLND) in stage Ⅳ low rectal cancer.

METHODS

We selected 71 consecutive stage Ⅳ low rectal cancer patients who underwent primary tumor resection, and enrolled 50 of these 71 patients without clinical LPLN metastasis. The patients had distant metastasis such as liver, lung, peritoneum, and paraaortic LN. Clinical LPLN metastasis was defined as LN with a maximum diameter

of 10 mm or more on preoperative pelvic computed tomography scan. All patients underwent primary tumor resection, 27 patients underwent total mesorectal excision (TME) with LPLND (LPLND group), and 23 patients underwent only TME (TME group). Bilateral LPLND was performed simultaneously with primary tumor resection in LPLND group. R0 resection of both primary and metastatic sites was achieved in 20 of 50 patients. We evaluated possible prognostic factors for 5-year overall survival (OS), and compared 5-year cumulative local recurrence between the LPLND and TME groups.

RESULTS

For OS, univariate analyses revealed no significant benefit in the LPLND compared with the TME group (28.7% *vs* 17.0%, $P = 0.523$); multivariate analysis revealed that R0 resection was an independent prognostic factor. Regarding cumulative local recurrence, the LPLND group showed no significant benefit compared with TME group (21.4% *vs* 14.8%, $P = 0.833$).

CONCLUSION

Prophylactic LPLND shows no oncological benefits in patients with Stage Ⅳ low rectal cancer without clinical LPLN metastasis.

Key words: Prophylactic lateral pelvic lymph node dissection; Stage Ⅳ; Low rectal cancer

Core tip: The clinical significance of prophylactic lateral pelvic lymph node dissection (LPLND) in stage Ⅳ low rectal cancer has not been proven. In this study, we showed two main findings concerning treatment strategy in these patients. First, prophylactic LPLND was not a significant prognostic factor for overall survival and did not contribute local control. Second, R0 resection was an independent prognostic factor for overall survival. These results suggest that prophylactic LPLND is not an important component of surgical treatment in stage Ⅳ low rectal cancer patients.

Tamura H, Shimada Y, Kameyama H, Yagi R, Tajima Y, Okamura T, Nakano M, Nakano M, Nagahashi M, Sakata J, Kobayashi T, Kosugi SI, Nogami H, Maruyama S, Takii Y, Wakai T. Prophylactic lateral pelvic lymph node dissection in stage Ⅳ low rectal cancer. *World J Clin Oncol* 2017; 8(5): 412-419 Available from: URL: http://www.wjgnet.com/2218-4333/full/v8/i5/412.htm DOI: http://dx.doi.org/10.5306/wjco.v8.i5.412

INTRODUCTION

In rectal cancer, lymphatic spread accords with the anatomical level of the tumor[1,2]. When the tumor is located above the peritoneal reflection, lymphatic cancer metastasis is predominantly associated with upward mesenteric spread along perirectal vessels originating from the inferior mesenteric artery. In contrast, when the tumor is located at or below the peritoneal reflection, lymphatic cancer metastasis can show upward mesenteric spread and lateral extramesenteric spread along the internal iliac vessels. Based on the rationale of lateral extramesenteric spread, lateral pelvic lymph node dissection (LPLND) is performed to eradicate LPLN metastasis in patients with rectal cancer located at or below the peritoneal reflection[3-9].

The management of LPLN associated with low rectal cancer differs considerably between Western countries and Japan. In Western countries, LPLN metastasis is generally considered as a metastatic disease, and preoperative chemoradiation and total mesorectal excision (TME) is the standard treatment[10]. In contrast, LPLN metastasis is regarded as a local disease in Japan, and TME with LPLND is performed for patients with locally advanced low rectal cancer[11]. Large-scale retrospective studies in Japan evaluated the survival outcome of patients with LPLN metastasis, and concluded that LPLN could be considered as regional lymph nodes in low rectal cancer[12].

LPLN metastasis was identified in approximately 20% of Japanese patients with T3 or T4 tumors who underwent LPLND[11,13]. Nevertheless, the clinical significance of LPLND has not been fully proven and a prospective study is needed to resolve whether LPLND has any survival benefit in patients with low rectal cancer. Accordingly, a randomized controlled trial was conducted to clarify the clinical significance of prophylactic LPLND for clinical stage Ⅱ and Ⅲ low rectal cancer (JCOG0212)[14]. However, to date, no studies have addressed the surgical outcome of TME with LPLND for stage Ⅳ low rectal cancer, and the clinical significance of LPLND for stage Ⅳ low rectal cancer is still unclear.

We retrospectively evaluated 50 consecutive stage Ⅳ low rectal cancer patients without clinical LPLN metastasis to assess the survival benefit of prophylactic LPLND in patients with stage Ⅳ low rectal cancer. We analyzed various prognostic factors including LPLND with respect to overall survival (OS), and evaluated cumulative local recurrence of patients with LPLND.

MATERIALS AND METHODS

Patients

We selected patients from our colorectal cancer databases with stage Ⅳ low rectal cancer according to the AJCC 7th edition[15], applied the following inclusion criteria: Adenocarcinoma confirmed on histological examination, preoperative pelvic computed tomography (CT) scan negative for clinical LPLN metastasis, and primary tumor resection undertaken at Niigata University Medical and Dental Hospital or Niigata Cancer Center Hospital between January 2000 and December 2015. We selected 71 consecutive stage Ⅳ low rectal cancer patients who underwent primary tumor resection, and enrolled 50 of these 71 patients without clinical LPLN

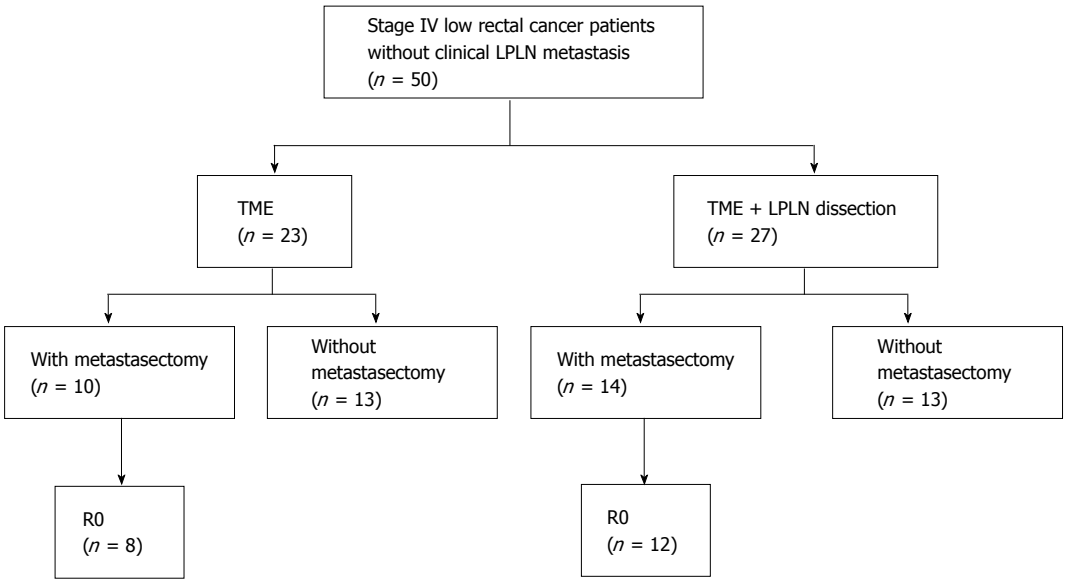

Figure 1 Flowchart of surgical treatment. TME: Total mesorectal excision; LPLN: Lateral pelvic lymph node.

metastasis (Figure 1, Table 1). All the patients had negative circumferential resection margin. Twenty of these 71 patients were excluded in the present study because they were diagnosed as positive for clinical LPLN metastasis by preoperative pelvic CT scan, and 1 patient was excluded because of loss of follow-up. Clinical LPLN metastasis was defined as LN with a maximum diameter of 10 mm or more on preoperative pelvic CT scan. In this study period, "therapeutic LPLND" was carried out for patients with clinical LPLN metastasis. For patients without clinical LPLN metastasis, whether "prophylactic LPLND" was performed or not was determined by preoperative conference. Neoadjuvant chemoradiotherapy (NACRT) was not administered at the participating institutions because it is uncertain whether this approach improves OS[16,17]. Distant metastasis was classified according to the JSCCR classification[18]. Liver metastases were classified into three categories (H1: 1-4 metastatic tumors all of maximum diameter 5 cm or less; H2: Those other than H1 or H3; H3: 5 or more metastatic tumors at least one of which has a maximum diameter of more than 5 cm). Lung metastases were classified into three categories (LM1: Metastasis limited to one lobe; LM2: Metastasis to more than one lobe in one side of lung; LM3: Metastasis to both sides of lungs). Peritoneal metastases were classified into three categories (P1: Metastasis localized to adjacent peritoneum; P2: Metastasis limited to distant peritoneum; P3: Diffuse metastasis to distant peritoneum). This retrospective study was performed in accordance with the Helsinki Declaration, and the Ethics Committee of the School of Medicine, Niigata University approved the study protocol (approval number: 2330), waiving patient consent.

Procedure of TME with LPLND and postoperative complications

Twenty-three patients underwent only TME ("TME group"), and 27 patients underwent TME with LPLND ("LPLND group"). Regarding LPLND, 26 procedures were performed as open surgery and 1 procedure was done as laparoscopic surgery. The LPLN were classified into five areas (distal internal iliac, proximal internal iliac, obturator, external iliac and common iliac) according to the JSCCR classification[18]. In the LPLND group, LPLND was carried out in accordance with previously reported methods[3,13,14]. Bilateral LPLND was performed simultaneously with primary tumor resection in LPLND group. Post-operative complications were monitored for 90 d after surgery and graded according to a standard classification[19]. Major complications were defined as grade $\geqslant$ 3.

Metastasectomy and residual tumor status

To achieve R0 resection of metastatic lesion, simultaneous or staged metastasectomy was planned according to the patients' condition. Essentially, simultaneous metastasectomy was performed when the patients had resectable intra-abdominal metastasis such as solitary liver metastasis which could be respected by partial hepatectomy, limited peritoneal dissemination, or paraaortic lymph nodes. Staged metastasectomy was planned when the patients had extra-abdominal metastasis such as lung metastasis, or liver metastasis which needed major hepatectomy such as right hepatic lobectomy. In this cohort, there were no patients who received conversion therapy such as hepatectomy for initially unresectable multiple liver metastasis. We classified the patients according to residual tumor status, *i.e.*, the patients who received R0 resection of both primary lesion and distant metastasis were classified as "R0", and the other patients in whom R0 resection could not be achieved were classified as "R2".

Prognostic factors

We evaluated possible prognostic factors including LPLND

for OS, and compared cumulative local recurrence rates between the TME and LPLND groups. To elucidate the factors influencing OS after surgery, 16 variables were tested in all 50 patients: Age (< 65 *vs* ⩾ 65 years), sex, preoperative Carcinoembryonic antigen (CEA) level (< 20 ng/mL *vs* ⩾ 20 ng/mL), tumor size (< 60 mm *vs* ⩾ 60 mm), T category (T2, 3 *vs* T4), histopathological grading (G1, 2 *vs* G3), lymphatic invasion (absence *vs* presence), venous invasion (absence *vs* presence), lymph node metastasis (absence *vs* presence), LPLND (absence *vs* presence), number of metastatic organs (1 *vs* 2), metastatic organ (liver only *vs* others), Grade 3 complication of primary tumor resection (absence *vs* presence), residual tumor status (R0 *vs* R2), Preoperative chemotherapy (absence *vs* presence), and Postoperative chemotherapy (absence *vs* presence).

Statistical analysis

After the operation, the patients were followed-up by physical examination, laboratory testing, and imaging. CEA and carbohydrate antigen 19-9 were monitored periodically. Disease recurrence and tumor progression were determined mainly by chest-abdominal-pelvic CT scans. Colonoscopy was performed to detect local recurrence at the anastomotic site. The median follow-up period of all 50 patients was 23.6 mo (range: 1-130). Statistical analyses were performed with IBM SPSS Statistics 22 (IBM Japan Inc., Tokyo, Japan). The relationships between each of the clinicopathological variables and residual tumor status were analyzed using Fisher's exact test. Five-year OS and cumulative local recurrence rates were estimated using the Kaplan-Meier method. The log-rank test was used to assess for significant difference between the subgroups by univariate analysis. To investigate independent prognostic factors for OS, factors with a *P* value of less than 0.10 in univariate analyses were entered into multivariate analysis. The Cox proportional hazards regression model was used to identify factors that were independently associated with OS after surgery. *P* values less than 0.05 were considered statistically significant.

RESULTS

Procedure and postoperative complications of primary tumor resection

All patients received R0 resection of primary site with the operative procedure as follows: 31 patients received low anterior resection, 18 patients received abdominoperineal resection, 1 patient received pelvic exenteration. Dysuria was observed in 20 patients, and all of them were grade 1 or 2. Major complications (grade ⩾ 3) were observed in 12 of 50 patients (24.0%); anastomotic leakage, surgical site infection, and anastomotic stenosis were observed in 4, 7, and 1 patients, respectively. Postoperative histopathological analysis revealed LPLN metastasis in 12 of 27 patients (44.4%) who received prophylactic LPLND, with a median number of 1 metastatic node per patient (range: 1-4). The sites of LPLN metastases were as follows: distal internal iliac nodes, proximal internal iliac nodes, obturator nodes, external iliac nodes, and common iliac nodes in 6, 5, 3, 1, and 1 patients, respectively.

Metastasectomy

Of the 50 patients, 24 received metastasectomy and 20 received R0 resection of both primary and metastatic sites (Figure 1). Sixteen patients simultaneously underwent primary tumor resection and metastasectomy. The details of the metastasectomy sites are as follows: Liver in 8 patients, limited peritoneal dissemination in 7 patients, and liver and paraaortic lymph node in 1 patient. Successful R0 resection was achieved in 14 of 16 patients; however, two patients who had liver and lung metastases underwent only hepatectomy because of progression of lung tumor after hepatectomy. In contrast, 8 patients underwent staged metastasectomy after primary tumor resection. The details of the metastasectomy sites are as follows: Liver in 4 patients, lung in 3 patient, liver and lung in 1 patient. R0 resection was achieved in 6 of these 8 patients; however, 1 patient who had liver and lung metastases underwent only hepatectomy because of progression of lung tumor after hepatectomy, and 1 patient who had lung metastasis underwent margin positive surgery. In contrast, 26 of 50 patients did not undergo metastasectomy because of tumor progression or development of new metastatic lesions after primary tumor resection.

Factors influencing OS after primary tumor resection

A comparison of clinicopathological characteristics between the LPLND and TME groups showed that there were no significant differences in 15 tested variables (Table 2). Five-year overall cumulative survival rates after primary tumor resection were 74.0% at 1 year, 43.7% at 3 years, and 23.4% at 5 years. Univariate analyses revealed that the LPLND group showed no significant benefit compared with TME group (28.7% *vs* 17.0%, *P* = 0.523) (Table 3 and Figure 2), and that age (⩾ 65 years) and R0 resection were factors whose *P* values were less than 0.10 for OS. Multivariate analysis identified R0 resection as significant independent prognostic factor for OS (*P* < 0.001) (Table 3).

Efficacy of prophylactic LPLND for local control

Five of the 50 patients showed local recurrence. The details of local recurrence sites are as follows: Anastomotic site in 2 patients, and the other intrapelvic space in 3 patients. One patient who had LPLND showed local recurrence of the right LPLN area. Twenty-seven patients with LPLND showed no significantly improved 5-year cumulative local recurrence rate compared with the 23 patients without LPLND (21.4% *vs* 14.8%, *P* = 0.833) (Figure 3).

DISCUSSION

In the present study, we showed that prophylactic

Table 1 Clinicopathological characteristics of the 50 patients

Variable	
Age (yr)[1]	58.5 (31-78)
Sex	
Male:female	43:7
Preoperative CEA level (ng/mL)[1]	24.5 (1.6–6856.5)
Tumor size (mm)[1]	63.0 (22–130)
T category	
T2:T3:T4	2:31:17
Histopathological grading	
G1:G2:G3	1:35:14
Lymphatic invasion	
Absence:Presence	6:44
Venous invasion	
Absence:Presence	10:40
Lymph node metastasis	
Absence:Presence	9:41
Pathological LPLN metastasis	
Absence:Presence	15:12
No. of metastatic organs	
1:2:3	44:5:1
Metastatic organ	
Liver:Lung:Peritoneum:Paraaortic LN:Bone	28:16:10:1:2
Grade of liver metastasis[2]	
H1:H2:H3	14:5:9
Grade of lung metastasis[2]	
LM1:LM2:LM3	8:7:1
Grade of peritoneal metastasis[2]	
P1:P2:P3	8:1:1
Grade ⩾ 3 Complication of primary tumor resection	
Absence:Presence	38:12
Residual tumor status	
R0:R2	20:30
Preoperative chemotherapy	
Absence:Presence	41:9
Postoperative chemotherapy	
Absence:Presence	7:43
Chemotherapy regimen	
5FU-LV and/or S-1 and/or capecitabine	25
FOLFOX and/or CapeOX and/or FOLFIRI	33
Bevacizumab	18
Cetuximab or panitumumab	4

[1]Data are expressed as median (range); [2]Distant metastasis was classified according to the Japanese Society for Cancer of the Colon and Rectum classification (See material and method). FOLFOX oxaliplatin, leucovorin, and 5FU, CapeOX oxaliplatin and capecitabine, FOLFIRI irinotecan, leucovorin, and 5FU. CEA: Carcinoembryonic antigen; LPLN: Lateral pelvic lymph node; LN: Lymph node; TME: Total mesorectal excision; 5FU: 5-Fluorouracil; LV: Leucovorin.

Table 2 Clinicopathological characteristics of patients in the lateral pelvic lymph node dissection and total mesorectal excision groups

Variable	TME group (*n* = 23)	LPLND group (*n* = 27)	*P* value
Age (yr)			
< 65	13	19	0.382
⩾ 65	10	8	
Sex			
Male	20	23	0.999
Female	3	4	
Preoperative CEA level (ng/mL)			
< 20	10	11	0.999
⩾ 20	13	16	
Tumor size (mm)			
< 60	7	8	0.999
⩾ 60	16	19	
T category			
T2, 3	11	14	0.999
T4	12	13	
Histopathological grading			
G1, 2	19	17	0.206
G3	4	10	
Lymphatic invasion			
Absence	3	3	0.999
Presence	20	24	
Venous invasion			
Absence	7	3	0.155
Presence	16	24	
Lymph node metastasis			
Absence	7	2	0.062
Presence	16	25	
No. of metastatic organs			
1	21	23	0.647
2, 3	2	4	
Metastatic organ			
Liver only	12	13	0.999
Others	11	14	
Grade ⩾ 3 complication of primary tumor resection			
Absence	17	21	0.999
Presence	6	6	
Residual tumor status			
R0	8	12	0.569
R2	15	15	
Preoperative chemotherapy			
Absence	18	23	0.715
Presence	5	4	
Postoperative chemotherapy			
Absence	4	3	0.689
Presence	19	24	

CEA: Carcinoembryonic antigen; LPLND: Lateral pelvic lymph node dissection; TME: Total mesorectal excision.

LPLND was not a significant prognostic factor for OS and did not contribute to local control. These results suggest that prophylactic LPLND is not an important component of surgical treatment in stage Ⅳ low rectal cancer.

It is possible that there are several acceptable treatment strategies in stage Ⅳ low rectal cancer patients without clinical LPLN metastasis. When the primary and metastatic sites are resectable, the patient can be treated with a staged or simultaneous resection to achieve R0 resection of both primary and metastatic sites. To achieve R0 resection of the primary site, the options are: (1) TME only; (2) TME with LPLND; (3) NAC followed by TME; and (4) NACRT followed by TME, *etc*. However, optimal treatment of patients with primary metastatic rectal cancer is controversial[20,21].

The NCCN guidelines state that NACRT is a standard treatment for stage Ⅱ/Ⅲ rectal cancer[10], however, it is also associated with increased toxicity (*e.g.*, radiation-induced injury, hematological toxicities). To date, the clinical significance of NACRT for stage Ⅳ low rectal cancer remains still unclear. van Dijk *et al*[20] reported that radical surgical treatment of all tumor sites carried out after short-course radiotherapy, and bevacizumab-

Table 3 Univariate and multivariate analyses of different prognostic factors for overall survival

Variable	Modality	*n*	Univariate		Multivariate	
			5-yr OS (%)	*P* value	HR (95%CI)	*P* value
Age (yr)	< 65	32	27.9	0.095	1	
	⩾ 65	18	14.8		1.5 (0.8–3.0)	0.197
Sex	Male	43	19.8	0.618		
	Female	7	42.9			
Preoperative CEA level (ng/mL)	< 20	21	23.7	0.671		
	⩾ 20	29	22.9			
Tumor size (mm)	< 60	15	29.6	0.634		
	⩾ 60	35	20.9			
T category	T2, 3	25	17.3	0.515		
	T4	25	32.5			
Histopathological grading	G1, 2	36	25	0.348		
	G3	14	21.4			
Lymphatic invasion	Absence	6	0	0.446		
	Presence	44	24.2			
Venous invasion	Absence	10	40	0.215		
	Presence	40	19.1			
Lymph node metastasis	Absence	9	0	0.904		
	Presence	41	27.5			
LPLND	Absence	23	17	0.523		
	Presence	27	28.7			
No. of metastatic organs	1	44	23.8	0.866		
	2	6	22.2			
Metastatic organ	Liver only	25	36	0.241		
	Others	25	10.6			
Grade ≥ 3 complication of primary tumor resection	Absence	38	28.8	0.398		
	Presence	12	9.5			
Residual tumor status	R0	20	59	< 0.001	1	
	R2	30	3.6		2.1 (1.4–3.0)	< 0.001
Preoperative chemotherapy	Absence	41	17.6	0.254		
	Presence	9	55.6			
Postoperative chemotherapy	Absence	7	38.1	0.397		
	Presence	43	24.3			

OS: Overall survival; CEA: Carcinoembryonic antigen; LPLND: Lateral pelvic lymph node dissection.

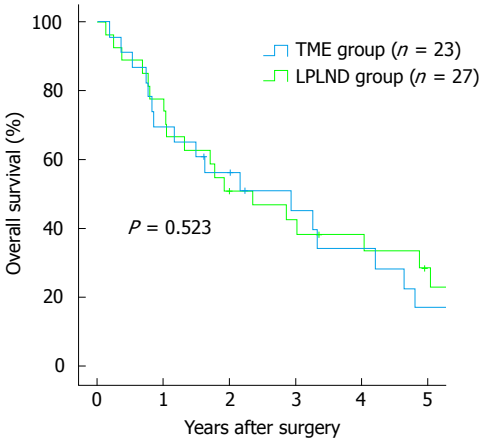

Figure 2 Comparative overall survival rates of patients with total mesorectal excision and lateral pelvic lymph node dissection groups. TME: Total mesorectal excision; LPLND: Lateral pelvic lymph node dissection.

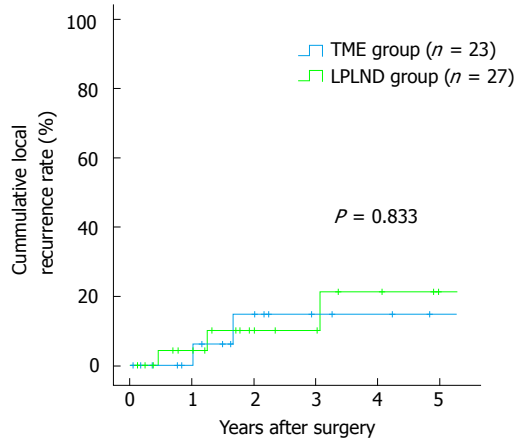

Figure 3 Comparative cumulative local recurrence rates of patients with total mesorectal excision and lateral pelvic lymph node dissection groups. TME: Total mesorectal excision; LPLND: Lateral pelvic lymph node dissection.

capecitabine-oxaliplatin combination therapy is a feasible and potentially curative approach in primary metastasized rectal cancer. Conversely, Butte *et al*[21] reported that selective exclusion of radiotherapy may be considered in rectal cancer patients who are diagnosed with simultaneous liver metastasis, because systemic

sites were overwhelmingly more common than pelvic recurrences after primary tumor resection. In stage Ⅳ patients, we surmised that subsequent metastasectomy and systemic chemotherapy are essential for cure; hence, a treatment strategy without NACRT could be a reasonable and acceptable approach to avoid the toxicity associated with NACRT.

To the best of our knowledge, this is the first report regarding the clinical significance of prophylactic LPLND in stage Ⅳ low rectal cancer patients without clinical LPLN metastasis. We demonstrated that prophylactic LPLND has no oncological benefits regarding OS and cumulative local recurrence in this setting. Previous studies reported that TME with LPLND is associated with significant morbidity, longer operative time, greater blood loss, and functional impairment, particularly impotence and bladder dysfunction[3,12-25]. To avoid the post-operative complications associated with LPLND and achieve early induction of postoperative chemotherapy, we think that prophylactic LPLND could be omitted for stage Ⅳ low rectal cancer patients without clinical LPLN metastasis.

We recognize several limitations in this study. First, this retrospective study included a small sample size. Second, we could not investigate how many patients, such as those who had multiple distant metastases, were excluded from the indications for primary tumor resection, because those patients were generally not referred to surgeons. Third, we could not investigate detailed parameters such as resectability criteria of distant metastases, comorbidity and response to chemotherapy. Fourth, it is possible that the LPLND group included patients with suspicious clinical LPLN metastasis of maximum diameter less than 10 mm, because histopathological LPLN metastases were observed in 12 of 27 patients (44.4%) patients in the LPLND group. Fifth, we included only patients without clinical LPLN metastasis. Hence, we could not assess the value of therapeutic LPLND for patients with clinical LPLN metastasis, and the clinical significance of LPLND for these patients is still unclear. In future, a multicenter prospective study is required to clarify the clinical significance of LPLND for stage Ⅳ low rectal cancer patients.

In conclusion, prophylactic LPLND shows no oncologic benefits in patients with stage Ⅳ low rectal cancer without clinical LPLN metastasis.

COMMENTS

Background

No studies have addressed the surgical outcome of total mesorectal excision with lateral pelvic lymph node dissection (LPLND) for stage Ⅳ low rectal cancer, and the clinical significance of LPLND for stage Ⅳ low rectal cancer is still unclear.

Research frontiers

There is little clinical information relating to LPLND for stage Ⅳ low rectal cancer.

Innovations and breakthroughs

This study is the first report regarding the clinical significance of prophylactic LPLND in stage Ⅳ low rectal cancer patients without clinical LPLN metastasis.

Applications

Prophylactic LPLND shows no oncologic benefits in patients with stage Ⅳ low rectal cancer without clinical LPLN metastasis.

Peer-review

To assess the clinical significance of prophylactic lateral pelvic lymph node dissection is the first research in stage Ⅳ low rectal cancer. The article is well-designed and important for clinical practice.

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CASE REPORT

First report of small cell lung cancer with PTHrP-induced hypercalcemic pancreatitis causing disconnected duct syndrome

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Informed consent statement: The patient discussed in this case report gave written consent to share imaging and discuss his medical information. This consent was witnessed.

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Abstract

Here we report a patient diagnosed with small cell lung cancer after first presenting with parathyroid hormone-related peptide-induced hypercalcemic pancreatitis and developed walled-off necrosis that resulted in disruption of the main pancreatic duct. Disconnected duct syndrome (DDS) is a rare syndrome that occurs when the main pancreatic duct exocrine flow is disrupted resulting in leakage of pancreatic enzymes and further inflammatory sequela. To date, no prior reports have described DDS occurring with paraneoplastic reactions. Diagnostic imaging techniques and therapeutic interventions are reviewed to provide insight into current approaches to DDS.

Key words: Disconnected duct syndrome; Parathyroid hormone-related peptide; Hypercalcemic pancreatitis

Core tip: Acute recurrent pancreatitis flares should raise concern for disconnected duct syndrome (DDS). This case is the first reported case of DDS caused by paraneoplastic hypercalcemia. Paraneoplastic syndromes may predispose patients to prolonged hypercalcemic pancreatitis and in turn, may predispose patients to DDS. Furthermore, this case report reviews the current approach and treatment difficulties of DDS as well as pancreatic walled-off necrosis.

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lung cancer with PTHrP-induced hypercalcemic pancreatitis causing disconnected duct syndrome. *World J Clin Oncol* 2017; 8(5): 420-424 Available from: URL: http://www.wjgnet.com/2218-4333/full/v8/i5/420.htm DOI: http://dx.doi.org/10.5306/wjco.v8.i5.420

INTRODUCTION

Disconnected duct syndrome (DDS) is a pancreatic syndrome where the main pancreatic duct is occluded and pancreatic exocrine flow leaks into the pancreatic parenchyma[1]. This syndrome frequently results in further inflammatory reactions such as sepsis, development of pseudocysts, and fistulizing disease. Etiologies of DDS are more commonly from mass-like lesions such as large pseudocysts, walled-off necrosis, or neoplasms obstructing the main pancreatic duct[1]. DDS often is difficult to treat due to narrow or complete occlusions requiring cannulation and increased surgical morbidity and mortality. Additionally, this case report discusses a unique cause of DDS and the current approaches used for diagnosis and treatment. To date, this is the first report of a DDS being related to a paraneoplastic syndrome.

CASE REPORT

A 38-year-old man with newly diagnosed small cell lung cancer (SCLC) presented in late July 2016 with acute onset epigastric pain, nausea, and vomiting. He was admitted one month prior for acute pancreatitis secondary to a calcium of 13.7 mg/dL (normal 8.4-10.3 mg/dL). He denied alcohol history or previous gall stones at that time, and imaging work up was only positive for pancreatic inflammation and a lung mass determined by biopsy to be SCLC. No evidence of bone metastasis was seen on imaging. During the July 2016 admission, vital signs at presentation were blood pressure 143/99 mmHg, heart rate 120 beat/min, respiratory rate 14 breaths/min, oxygen saturation 100%, temperature 36.6 ℃, and physical exam was only positive for epigastric tenderness. Labs demonstrated a serum lipase of 2030 U/L (normal < 90 U/L), serum calcium of 11 mg/dL (normal 8.4-10.3 mg/dL), parathyroid hormone less than 9 pG/mL (normal 12-65 pG/mL) and parathyroid-related peptide of 3.9 pmol/L (normal < 2 pmol/L). Triglycerides were normal. Abdominal ultrasound revealed no evidence of gallstones. MRI of the abdomen with magnetic resonance cholangiopancreatography (MRCP) showed multiple cystic areas with rim enhancement replacing large portions of the pancreatic body with the largest centered in the mid-body of the pancreas measuring 3.5 cm × 6.2 cm compressing the main pancreatic duct as well as a 2 cm × 4.3 cm collection extending into the pancreatic groove (Figure 1). MRCP displayed complete lack of enhancement of the main pancreatic duct (Figure 2). A diagnosis of DDS was made based off of these findings. Development of the walled-off necrosis and pancreatic inflammation was thought to be secondary to repeated paraneoplastic-induced pancreatitis episodes. ERCP-guided cannulation of main pancreatic

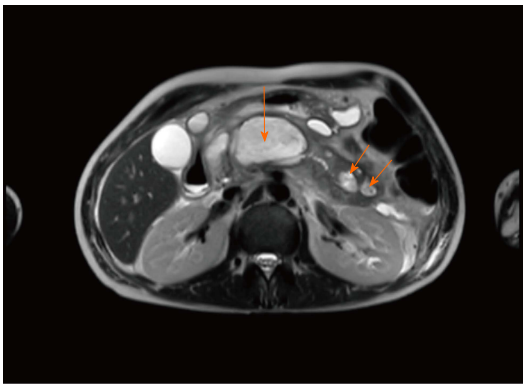

Figure 1 Magnetic resonance imaging of abdomen with and without contrast during July 2016 presentation. Image displays large walled-off necrosis within the body and tail of the pancreas (arrows).

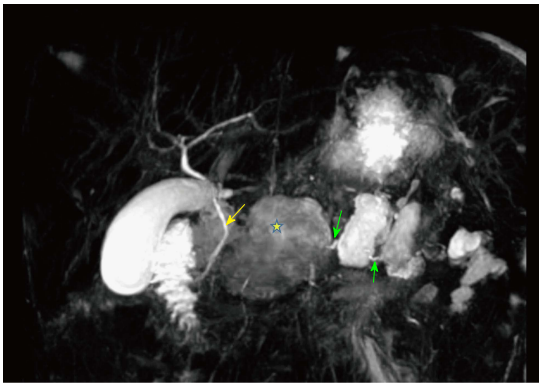

Figure 2 Magnetic resonance cholangiopancreatography performed during July 2016 admission. Image displays poorly defined main pancreatic duct (green arrows) throughout the pancreas. Common bile duct defined well (yellow arrow) with lack of contrast accentuating the main pancreatic duct. A large walled-off necrosis well imaged again (star).

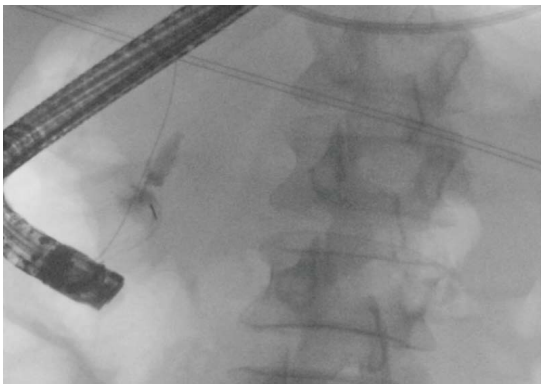

Figure 3 Endoscopic retrograde cholangiopancreatography performed during July 2016 admission. Image displays failure of contrast dye to define pancreatic duct and failure of guidewire to cannulate pancreatic duct. Guidewire continues to be diverted to common bile duct which provides evidence of pancreatic duct obstruction.

Table 1 Definitions and descriptions of structural complications of acute pancreatitis

Structural complications of acute pancreatitis[2]	
Acute peripancreatic fluid collection	Defined as peripancreatic fluid within the first 4 wk of interstitial edematous pancreatitis Homogeneous collection with fluid density No visible encapsulating wall around fluid collection Adjacent to pancreas
Pancreatic pseudocyst	Defined as an encapsulated fluid collection usually forming > 4 wk from initial pancreatitis event with visible inflammatory wall typically outside the pancreas with minimal or no necrotic features forming Homogeneous fluid density with no non-liquid components
Acute necrotic collection	Defined as a fluid collection with variable amounts of fluid and necrosis without a visible encapsulating wall Only can occur with necrotizing pancreatitis Can involve pancreatic parenchyma and/or peripancreatic tissue Heterogeneous and non-liquid density of varying degrees
Walled-off necrosis	Defined as a mature collection of pancreatic and/or peripancreatic necrosis with an encapsulating inflammatory wall typically requiring > 4 wk from initial pancreatitis to form Only can occur with necrotizing pancreatitis Heterogeneous with liquid and non-liquid density with varying degrees of loculation

duct past the pancreatic head was unsuccessful due to complete occlusion of the duct (Figure 3). Pancreatic duct stent placement was unsuccessful. Endoscopic ultrasound visualized the walled off necrosis, but transmural drainage was avoided due to symptomatic improvement with conservative management. The patient was managed conservatively with pain management and bisphosphonates over the following 24 wk until cholecystectomy and surgical necrosectomy were performed. The surgery was uncomplicated. Currently, the patient was transitioned to home hospice due to progression of his cancer.

DISCUSSION

Acute pancreatitis is defined by the Atlanta Classification as having: (1) Typical pain; (2) imaging showing pancreatic inflammation; and (3) elevation in amylase or lipase > 3 × the upper limit of normal. Two of the three criteria must be present to confirm the diagnosis[2]. Acute pancreatitis can be complicated by the formation of fluid collections which have been defined and characterized by the 2012 revised Atlanta Classification (Table 1)[2]. Major distinguishing features of fluid collections are the required time for formation, the presence of an encapsulating inflammatory wall, and heterogeneity[2]. An acute peripancreatic fluid collection (APFC) is a collection of adjacent fluid that develops within the first four weeks of the initial pancreatitis[2]. APFC is not contained by a visible encapsulating inflammatory wall and is a homogeneous collection with fluid density[2]. In contrast, a pancreatic pseudocyst is a fluid collection usually outside of the pancreas and typically requires four weeks or more to develop[2]. A pseudocyst has a visible encapsulating inflammatory wall and is homogeneous with only fluid components[2]. If acute pancreatitis progresses to necrotizing pancreatitis, an acute necrotic collection (ANC) can develop. An ANC develops usually less than four weeks from initial event and does not have visible encapsulating walls. ANC can be distinguished from an APFC by a heterogeneous appearance from localized liquid and necrotic pancreatic tissue. After approximately four weeks, an ANC will develop an encapsulated inflammatory wall which is termed a walled-off necrosis (WON). A WON will continue to have a heterogeneous appearance from accumulated fluid and necrotic pancreatic tissue[2].

Acute recurrent pancreatitis is a clinical condition that is defined as two or more attacks of pancreatitis without evidence of underlying chronic pancreatitis[3]. Acute recurrent pancreatitis is often attributed to gallstones, alcohol ingestion, or idiopathic causes[3]. Furthermore, acute recurrent pancreatitis can progress to necrotizing pancreatitis and develop inflammatory fluid collections that obstruct pancreatic duct drainage, termed DDS. DDS should be considered on a differential diagnosis particularly when a patient presents with repeated bouts of pancreatitis and enlarging pancreatic fluid collections. DDS is a syndrome that starts with an episode of acute pancreatitis that typically develops a large fluid collection or necrosis. This initial fluid collection results in compression of the main pancreatic duct. Disruption of the main pancreatic duct flow, most commonly in the neck or body of the pancreas[4], results in blockage and leakage of distal drainage of pancreatic enzymes. Leakage of these enzymes into the pancreatic parenchyma results in further inflammatory sequela such as more fluid collections, fistulas, or sepsis. Causes of DDS all result in a significant narrowing or complete occlusion of the main pancreatic duct. More frequently encountered causes of duct obstruction are large pseudocysts, necrotic lesions, trauma, and abdominal neoplasms[4,5]. Less commonly, causes such as intra-ductal pancreatic mucinous neoplasm or calculi can result in DDS. Of note, acute recurrent pancreatitis as a presenting feature of SCLC is rare and if present, pancreatitis is more commonly from metastatic lesions obstructing the pancreatic duct rather than PTHrP-induced hypercalcemia[6]. Hypercalcemia results typically from either elevated PTHrP production or osteolytic activity from bone metastasis. Paraneoplastic

hypercalcemia is most commonly associated with squamous cell carcinoma of the lung as opposed to small cell lung cancer[7]. The presence of paraneoplastic hypercalcemia in lung cancer has been associated with poorer survival outcomes[8]. No previous cases have reported DDS developing from PTHrP-induced hypercalcemic pancreatitis. There is no clear consensus on which cause of pancreatitis is most likely to result in DDS. This patient possessed persistent hypercalcemia and an aggressive malignancy, both risk factors for pancreatitis, and in turn, risks for development of DDS.

Patients presenting with acute pancreatitis will often have already received an abdominal ultrasound and/or CT abdomen with and without contrast in the emergency department to visualize causes of pancreatic inflammation. In patients with suspected DDS, MRCP is particularly useful by providing detailed mapping of the pancreaticobiliary ducts[4]. ERCP is no longer routinely used for diagnostic purposes as MRCP can provide the same information without the risks associated with ERCP, but is undertaken with therapeutic intentions such as relieving obstructions *via* stent placement and displaying resolution of obstruction on repeat fluoroscopy[9]. Additionally, endoscopic ultrasound (EUS) is utilized for more accurate visualization of the pancreatic duct and ultrasound-guided drainage of large fluid collections causing obstruction of the duct[10]. For cases of DDS involving WON, endoscopic necrosectomy can be coupled with EUS to relieve obstructions by debriding and opening necrotic septa through gastric or duodenum access[11].

Whenever possible, definitive intervention is delayed 4 wk or more to allow organization of necrotic collections and development of an encapsulating wall[12]. Initially, if the patient is clinically stable, a minimally invasive approach can be performed to relieve ductal compression with endoscopic/percutaneous approaches favored over open surgical necrosectomy[12,13]. If endoscopic interventions are unsuccessful, surgical intervention (*i.e.* necrosectomy, Roux-en-Y, or debridement) is required to relieve obstructions. While data suggests that minimally invasive approaches are superior to surgical intervention for necrosectomy, whether endoscopic or surgical intervention is superior for DDS is still a subject of debate[14,15]. For DDS, endoscopic intervention is typically first-line and less invasive than surgery, but success is dependent on cannulation of narrow strictures and stent placement in cases of ERCP and optimal positioning of lesions for drainage in cases of EUS[14]. Surgical interventions are often successful at relieving obstructions, but often are associated with higher morbidity and mortality compared to endoscopy[11,13,15]. This case demonstrates the approach to a unique case of DDS and highlights the difficulty associated with treatment of DDS. Additionally, this case is evidence of the importance of earlier detection of lesions prior to complete ductal obstructions. In complete pancreatic duct obstructions, ERCP efficacy may be limited and result in patients having to undertake greater morbidity and mortality risks to relieve obstructions.

COMMENTS

Case characteristics

A 38-year-old man with small cell lung cancer presented with acute onset epigastric pain, nausea and vomiting.

Clinical diagnosis

Tenderness in the epigastric region of the abdomen and tachycardia.

Differential diagnosis

Acute hypercalcemic pancreatitis, acute recurrent pancreatitis, gastric ulcer, erosive gastropathy, cholelithiasis, choledocholithiasis.

Laboratory diagnosis

Labs demonstrated lipase 2030 U/L (normal < 90 U/L), serum calcium of 11 mg/dL (normal 8.4-10.3 mg/dL), parathyroid hormone less than 9 pG/mL (normal 12-65 pG/mL), parathyroid-related peptide of 3.9 pmol/L (normal < 2 pmol/L), and normal triglycerides.

Imaging diagnosis

Magnetic resonance imaging with magnetic resonance cholangiopancreatography showed multiple cystic areas with rim enhancement replacing large portions of the pancreatic body with the largest centered in the mid-body of the pancreas measuring 3.5 cm × 6.2 cm compressing the main pancreatic duct as well as a 2 cm × 4.3 cm collection extending into the pancreatic groove.

Treatment

Unsuccessful endoscopic retrograde cholangiopancreatography-guided main pancreatic stent placement followed by successful surgical necrosectomy and cholecystectomy.

Related reports

Disconnected duct syndrome (DDS) is rare syndrome that often presents with recurrent pancreatitis flares. The syndrome is more commonly caused by mass lesions obstructing the main pancreatic duct. Paraneoplastic hypercalcemia is more often associated with squamous cell lung cancer as opposed to small cell lung cancer.

Term explanation

DDS is a pancreatic syndrome where the main pancreatic duct is occluded and pancreatic exocrine flow leaks into the pancreatic parenchyma. This syndrome frequently results in further inflammatory reactions such as sepsis, development of pseudocysts, and fistulizing disease.

Experiences and lessons

Acute recurrent pancreatitis should raise concerns for DDS due to exocrine leakage into pancreatic parenchyma causing repeated inflammatory reactions. Although less common than squamous cell lung cancer, small cell lung cancer can result in paraneoplastic hypercalcemia which can expose patients to prolonged risks of pancreatitis. This prolonged risk of pancreatitis may increase the risk for develop of DDS.

Peer-review

This is an interesting case for physician.

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CASE REPORT

Charcot-Marie-Tooth hereditary neuropathy revealed after administration of docetaxel in advanced breast cancer

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Abstract

Charcot-Marie-Tooth (CMT) neuropathy is the most common hereditary cause of neuropathy. Diagnosis is usually not made during the childhood but in adolescence or late adulthood. It is reported in the literature that some neurotoxic chemotherapeutical agents can reveal an asymptomatic CMT IA hereditary neuropathy. To our knowledge, we report here the first case of CMT IA revealed in a 55-year-old woman after the administration of docetaxel/trastuzumab/pertuzumab for metastatic breast cancer. This case stresses again the necessity to obtain a complete personal and familial anamnesis and to perform a neurologic examination before the administration of neurotoxic chemotherapeutical agents to prevent the clinical expression of these hereditary neuropathies.

Key words: Charcot-Marie-Tooth IA; Docetaxel; Breast cancer; Neurotoxicity; Peripheral neuropathy

Core tip: This case report represents the first case of Charcot-Marie-Tooth IA revealed after the administration of docetaxel/trastuzumab/pertuzumab for metastatic breast cancer. This paper will help to focus on the revelation of rare hereditary neuropathies after the administration of chemotherapies.

Kourie HR, Mavroudakis N, Aftimos P, Piccart M. Charcot-Marie-Tooth hereditary neuropathy revealed after administration of

docetaxel in advanced breast cancer. *World J Clin Oncol* 2017; 8(5): 425-428 Available from: URL: http://www.wjgnet.com/2218-4333/full/v8/i5/425.htm DOI: http://dx.doi.org/10.5306/wjco.v8.i5.425

INTRODUCTION

Charcot-Marie-Tooth (CMT) type Ⅰ neuropathy is the most common hereditary cause of neuropathy. Seventy percent to 80% of these patients present the subtype IA. This disease involves the motor and sensory peripheral nerves. Age of onset is variable; diagnosis is usually made between the age of 5 and 25 years. The diagnosis of CMT IA is confirmed with genetic testing and electro-diagnostic studies. There is no approved medical therapy to prevent the progression of CMT[1].

To our knowledge, we report here the first case of CMT IA revealed in a patient after receiving docetaxel/trastuzumab/pertuzumab for metastatic breast cancer. We first discuss the originality of our case and the potential role of chemotherapies in revealing or accentuating these syndromes. Then, we review the reported cases in the literature describing the relationship between these diseases and different chemotherapeutical agents.

CASE REPROT

We present the case of a 55-year-old patient, with unremarkable past medical history, who was diagnosed with HER2-positive and hormonal receptor positive stage Ⅳ metastatic breast cancer in 2014 with a primary tumor of 2.7 cm and one liver metastasis. Her first-line therapy consisted of the standard of care regimen docetaxel combined to trastuzumab and pertuzumab followed by surgery of the primary breast tumor and radiofrequency ablation of the unique metastatic hepatic lesion given an excellent response and the presence of oligometastatic disease.

Chemotherapy was stopped after 6 cycles of docetaxel and the patient was kept on maintenance therapy with trastuzumab and pertuzumab. Three months after the last cycle of chemotherapy, the patient developed numbness in the extremities, generalized depressed tendon reflexes, and hypoesthesia in the lower third of legs. At the clinical examination, pes cavus foot deformity, bilateral foot drop and generalized depressed tendon reflexes were detected.

After a complete anamnesis, the patient mentioned that many members of her family (her sister, her niece and her grand-father) were diagnosed with the CMT type IA disease. A genetic analysis of *PMP22* gene confirmed the diagnosis in our patient and conduction velocity studies demonstrated demyelinating abnormalities concordant with the diagnosis. In fact, multiple ligation-dependent probe amplification of the exons 1-5 of the *PMP22* gene located at 17p11.2 showed genomic duplication comprising the *PMP22* gene.

The patient continued her treatment for breast cancer based on targeted therapies (pertuzumab and trastuzumab) and hormonal therapy (letrozole) and is currently in complete remission. For her stable persistent neurologic deficits physiotherapy was prescribed for maintaining posture and balance, genetic counseling for the family members, namely her two sons, and avoidance of neurotoxic drugs.

DISCUSSION

Neurologic toxicities represent the second most frequent chemotherapy-induced side effect after hematologic toxicities. Vinca-alkaloids, taxanes and platinum-based agents are the most frequent drugs inducing peripheral neurotoxicity. These drugs are used in the treatment of ovarian, breast, lung, prostate, colon and hematological malignancies. Chemotherapy-induced neuropathy is usually dose-dependent. Patients with pre-existing neuropathic symptoms due to diabetes, hereditary neuropathies or earlier treatment with neurotoxic chemotherapy are probably more vulnerable to further development of chemotherapy-induced peripheral neuropathy[2,3].

We report, here, an interesting case of a hereditary CMT IA disease revealed after the administration of docetaxel in combination with anti-HER2 antibodies, in an advanced HER2-positive breast cancer. Our case represents the first case of CMT IA revealed after the administration of docetaxel. It is less likely that this disease was revealed by trastuzumab or pertuzumab, because it was not reported in the literature any CMT related to biologic agents.

A case of an aggravation of CMT after the administration of carboplatin and paclitaxel for ovarian cancer was reported before; the symptomatology resolved after the replacement of paclitaxel by docetaxel, considered as less neurotoxic 4. Thus, our case demonstrates that even less neurotoxic taxanes, as docetaxel, can sometimes reveal or worsen CMT neuropathies.

In some cases, CMT IA has been diagnosed after the administration of neurotoxic drugs including chemotherapeutical agents, mainly vincristine. The cases reported in the literature are summarized in Table 1.

A retrospective case series, in three families with known hereditary neuropathies treated with vincristine, concluded that vincristine in patients with 17p11.2-12 may lead to severe neurotoxicity from vincristine and that this drug should not be administered in patients with CMT1A[5]. On the other hand, a recent study of the Mayo Clinic investigated the association of non-CMT polyneuropathy with *CMT* genes in patients treated with paclitaxel. The results demonstrated a relationship between the *CMT* gene allelic variability and the susceptibility to chemotherapy-induced peripheral neuropathy[6].

This case stresses again the importance for on-

Table 1 Summary of all the reported cases in the literature of Charcot-Marie-Tooth revealed after administration of chemotherapy

Ref.	Patient characteristics	Drug	Malignancy	Signs and symptoms	Diagnosis
Uno *et al*[7], 1999	44 M	Vincristine	NHL	Rapid and marked weakening progressing to quadriplegia and bulbar palsy pes cavus (hollow foot)	Slower nerve conduction velocity 17p11.2-12 duplication
Martino *et al*[4], 2005	F	Paclitaxel/ Carboplatine	Ovarian cancer	Distal sensory and motor neuropathy; Unable to walk, write, or drive	Already diagnosed
Hildebrandt *et al*[8], 2000	52 F	Vincristine	NHL	Dysphagia, dysarthria, muscular weakness of both lower and upper extremities, areflexia, paraesthesia of the fingertips and bilateral sensory impairment of feet and lower legs	Peripheral axonal and demyelinating sensorimotor neuropathy 17p11.2 duplication
Graf *et al*[5], 1996	9 F	Vincristine	Acute lymphoblastic leukemia	Severe acquired weakness, areflexia and distal muscle atrophy	17p duplication
	18 F		Burkitt lymphoma	Pes cavus, distal muscle atrophy and weakness, stocking glove sensory deficits	17p duplication Slower nerve conducting velocity
	46 M		Testicular embryonal cell carcinoma	Foot drop, per cavus and areflexia, marked weakness	Slow motor nerve conduction velocity

NHL: Non-Hodgkin lymphoma; M: Male; F: Female.

cologists to perform a complete anamnesis on past personal and familial history, before the administration of a neurotoxic chemotherapeutical regimen. It is also crucial to perform a complete neurological examination before administrating neurotoxic chemotherapies to avoid the worsening of non-diagnosed peripheral neuropathies.

COMMENTS

Case characteristics

The patient presented numbness in the extremities, generalized depressed tendon reflexes, and hypoesthesia in the lower third of legs, 3 mo after the last cycle of chemotherapy.

Clinical findings

The clinical examination of the patient revealed pes cavus foot deformity, bilateral foot drop and generalized depressed tendon reflexes.

Differential diagnosis

Acute myelitis or Guillain-Barré syndrome are possible differential diagnosis.

Laboratory findings

A multiple ligation-dependant probe amplification of the exons 1-5 of the *PMP22* gene located at 17p11.2 showed genomic duplication comprising the *PMP22* gene.

Pathological diagnosis

Pathological diagnosis was not necessary.

Treatment

Physiotherapy was prescribed for maintaining posture and balance, genetic counseling for the family members, namely her two sons, and avoidance of neurotoxic drugs.

Experiences and lessons

Perform a complete anamnesis on past personal and familial history, before the administration of a neurotoxic chemotherapeutical regimen. Perform a complete neurological examination before administrating neurotoxic chemotherapies to avoid the worsening of non-diagnosed hereditary peripheral neuropathies.

Peer-review

This manuscript presents the first case of Charcot-Marie-Tooth disease identified after the administration of docetaxel in combination with anti-HER2 antibodies. This is an important and interesting report of a rare case.

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