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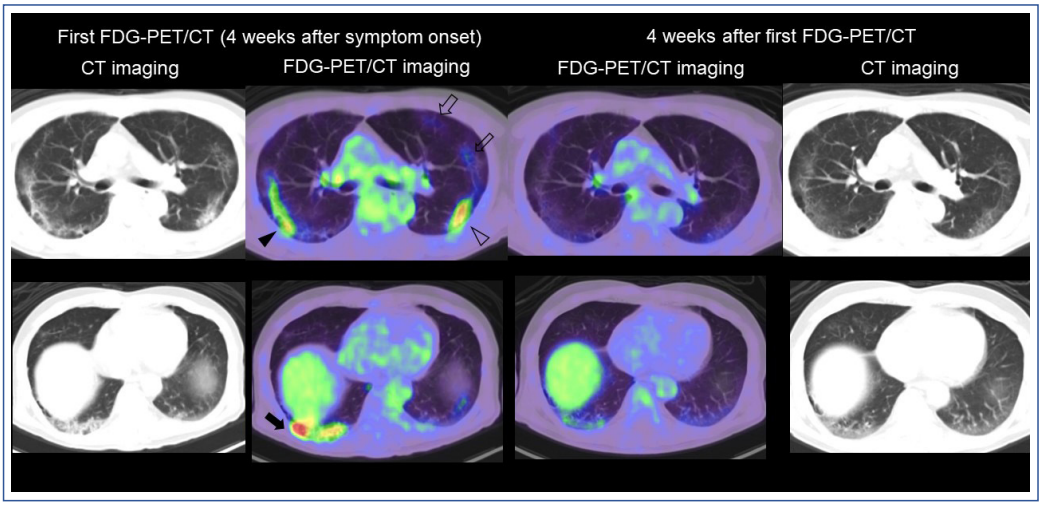

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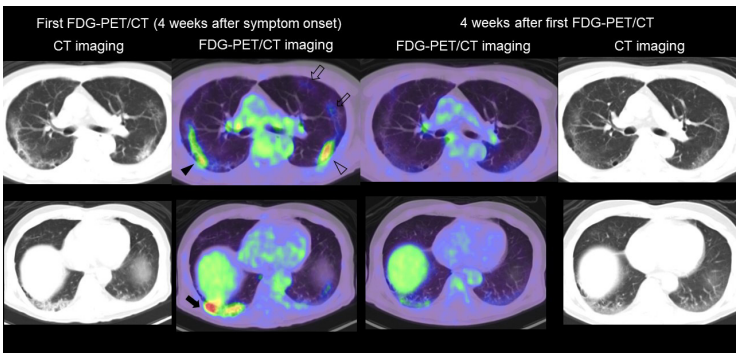

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Pancreatic islet transplantation: toward definitive treatment for diabetes mellitus

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Abstract: Since the late 20th century, advances in pancreatic islet transplantation have targeted improved glycemic control and fewer hypoglycemic events in patients with type 1 diabetes, and some important milestones have been reached. Following the Edmonton group's success in achieving insulin independence in all transplanted patients with type 1 diabetes, clinical islet transplantation is now performed worldwide. β cell replacement therapy for type 1 diabetes was established based on the favorable outcomes of a phase 3, prospective, open-label, single-arm, clinical study conducted at 8 centers in North America, in which 42 of 48 patients who underwent islet transplantation from 2008 to 2011 achieved HbA1c < 7.0% (53 mmol/mol) at day 365, which was maintained at 2 years in 34 patients. In Japan, a phase 2 multicenter clinical trial of islet transplantation for type 1 diabetes patients is currently ongoing and will end soon, but the interim results have already led to positive changes, with allogeneic islet transplantation being covered by the national health insurance system since April 2020. Current efforts are being made to solve the problem of donor shortage by studying alternative donor sources, such as porcine islets and pancreatic progenitor cells derived from pluripotent stem cells. The results of clinical trials in this area are eagerly awaited. It is hoped that they will contribute to establishing alternative sources for insulin-producing β cells in the near future.

Keywords: type 1 diabetes, pancreas preservation, islet isolation, islet purification, instant blood-mediated inflammatory reaction, immunosuppression

Introduction

Whole pancreas transplantation has been performed since 1966 to facilitate endogenous insulin secretion and effectively manage blood glucose, but the mortality rate after 5 years still exceeds 10% (*1*). Pancreatic islet isolation techniques have been developed to provide a less invasive procedure (*2*) and a number of important milestones have been reached in its development. Islet transplantation was begun in the early 1970s using rat models (*3*), and the liver was considered to be the most appropriate site of engraftment *via* portal vein infusion (*4*). The first human islet transplantation was reported in 1977 in a preliminary report (*5*). Isolated islets from deceased donors were transplanted into the peritoneal cavity and portal vein of at least 7 diabetic patients, but none of them achieved insulin independence (*6*). In 1980, it was reported that 10 patients with chronic pancreatitis and unbearable pain underwent islet autotransplantation accompanied by total or distal pancreatectomy, with some patients successfully avoiding pancreatogenic diabetes after surgery (*7*). This confirmed that transplanted islets could indeed function as intended, but it was also clear that it can be difficult to adequately control rejection, inflammatory and autoimmune responses.

After methods were developed to isolate human pancreatic islets (*8*), in 1990 Scharp *et al.* reported insulin independence in a type 1 diabetes patient following allogeneic islet transplantation (*9*). A decade later, the Edmonton group, led by James Shapiro, reported that all 7 of their type 1 diabetes patients achieved insulin independence after allogeneic islet transplantation from an average of 2 donors using glucocorticoid-free immunosuppressants (*10*). Then, in 2005, the University of Minnesota group led by Bernard Hering reported that all 8 of their type 1 diabetes patients achieved insulin independence with transplantation from a single donor (*11*). This advancement led to a multicenter, phase 3, prospective, open-label, single-arm study being conducted at 8 centers in North America with publication of the results in 2016 (*12*). Between 2008 and 2011, 48 type 1 diabetes patients without stimulated C-peptide secretion had received islets from brain-dead donors; 42 patients achieved a HbA1c < 7.0% (53 mmol/mol) at day 365, and 34 maintained this level at 2 years with a marked reduction in severe hypoglycemia.

Based on the results of this trial, islet transplantation is now recognized as a standard treatment for unstable type 1 diabetes. A multicenter randomized controlled trial with type 1 diabetes patients in Europe later confirmed the safety and efficacy of islet transplantation compared with intensive insulin therapy (*13*). Other clinical trials have been completed or are underway (*14*), and bring promise of further developments in islet transplantation.

Here, we review some of the major achievements made thus far in pancreatic islet transplantation, including changes in islet preparation and improvements in immunosuppressive drugs. We discuss the current state of islet transplantation in Japan, and we consider future prospects using alternative islet sources and whether such developments can bring a definitive treatment for diabetes mellitus closer.

Transplant recipients

Islet allotransplantation has been performed in patients with brittle type 1 diabetes characterized by unstable glycemic control with frequent and unpredictable severe hypoglycemic episodes that cannot be controlled by optimal treatment from diabetologists. Unlike exogenous insulin, islets can physiologically produce and release insulin, resulting in strict blood glucose control. Current evidence-based guidelines propose a four-stage algorithm to treat type 1 diabetes, recommending that islet or pancreas transplantation be considered at the last stage in patients with persistent problematic hypoglycemia, defined as 2 or more episodes per year of severe hypoglycemia or as 1 episode associated with impaired awareness of hypoglycemia, extreme glycemic lability, or major fear and maladaptive behavior (*15*). If the recipient experiences severe chronic renal failure and requires a kidney transplant, a simultaneous kidney-islet transplantation or simultaneous pancreas-kidney transplantation is recommended. However, it is unclear if an islet transplantation should be performed in a patient with problematic hypoglycemia and an intermediate estimated glomerular filtration rate (eGFR) (30-60 mL/min) because the subsequent life-long administration of immunosuppressants might increase the risk of end-stage renal failure. Immunosuppression for the rest of the recipient's life also increases the risk of severe infections and malignancies. It is thus important to screen for endogenous infections and cancers (*16*).

Islet preparations

Pancreas preservation

In 2000, it was determined that single-donor islet transplants were viable after cold preservation of donor pancreases stored for less than 8 to 10 h, even in University of Wisconsin solution (UWS), a standard preservation solution for pancreas transplantation (*17*). A decade earlier, Kuroda *et al.* (*18*) reported that perfluorochemical (PFC), used as an oxygen-supplying agent for the cold storage of canine pancreas in Euro-Collins solution, enabled preservation up to 48 h in the two-layer cold storage apparatus, as tested by subsequent pancreas autotransplantation. Tanioka *et al.* (*19*) applied a UWS/PFC two-layer method (TLM) to preserve dog pancreas for 24 h before islet isolation, resulting in a huge increase in islet equivalents per gram pancreas compared with conventional simple cold storage in UWS. Hering *et al.* (*11,20*) used TLM for clinical islet transplants, but with less than 8 h of cold storage time. A few years later, two large-scale studies and a meta-analysis showed no beneficial effect of TLM on islet isolation and transplantation outcomes compared with UWS alone (*21-23*). However, several other meta-analyses reported that use of TLM produced a significantly higher islet yield (*24-26*). Moreover, a meta-analysis revealed that the beneficial effects of TLM on islet yield were correlated with a prolonged (> 20 h) cold ischemic time (*26*), which had been suggested by the previous meta-analysis (*23*).

As for the pancreas preservation solution, UWS had been used since the 1980s for clinical islet transplants (*27*) but had several disadvantages, including instability, high cost due to its short expiration date, and high viscosity. Noguchi *et al.* (*28*) reported increased islet yield from pancreases preserved using a TLM comprising extracellular-type trehalose-containing Kyoto (ET-Kyoto) solution with a trypsin inhibitor, namely, M-Kyoto solution. They subsequently found a higher viability of isolated islets with ulinastatin as the trypsin inhibitor than with gabexate mesilate or nafamostat mesilate (*29*).

To achieve a satisfactory distribution of collagenase in islet isolation, Sawada *et al.* (*30*) examined the importance of UWS injection into the rat pancreatic duct at the time of procurement, which protects the ducts from cold ischemic injury, leading to the clinical application of ductal preservation using M-Kyoto solution (*31*).

Islet isolation

The isolation of islets by incubation with collagenase originated from Moskalewiski's 1965 study in guinea pigs (*32*). For larger animals, a method for the preparation of viable islet cells from healthy dog pancreas was described in 1981, based on perfusion of the pancreatic duct with higher concentrations of collagenase, which resulted in improved islet yield and decreased acinar contamination (*33*). A method developed for the isolation of islets of Langerhans from the human pancreas was reported in 1984; this technique was based on injection of a high concentration of collagenase into the pancreatic duct under pressure, followed by a short incubation at 39°C (*34*). Finally, Ricordi *et al.* (*8*) published a semiautomated method

for the isolation of human pancreatic islets, leading to the first case of allogeneic islet transplantation for type 1 diabetes, which achieved a short period of insulin independence (*9*). The dissociation chamber at the core of the automated method was named the Ricordi chamber after Dr. Camillo Ricordi. The Ricordi Automated Method has since become the gold standard for human and large animal pancreatic processing, contributing to the success of clinical trials of islet transplantations and an increasing number of such trials worldwide (*35*).

Although the variable enzymatic composition of crude collagenases undermined the reproducibility of islet isolation procedures, Wolters *et al.* (*36*) found that only collagenase class I (CC-I), collagenase class II (CC-II), and a supplementary protease are essential for effective pancreas digestion, leading in 1995 to a commercially available purified enzyme blend, Liberase HI (Roche, Indianapolis, IN), which helped to significantly increase the number of human islet allotransplantations. In addition, Brandhorst *et al.* (*37*) revealed that a delicate ratio between CC-II and CC-I could reduce the demand on supplementary proteases, which has significant implications for the viability of isolated islets. Although Liberase HI was very effective, it was withdrawn from the market in 2007 because of the risk for potential transmission of bovine spongiform encephalopathy due to the use of bovine neural tissue during its production.

Shimoda *et al.* (*38*) examined various collagenases as alternatives to Liberase HI, such as collagenase NB1 (Serva, Heidelberg, Germany), Clzyme Collagenase HA (Vitacyte, Indianapolis, IN), and mammalian tissue-free Liberase MTF (Roche), and showed that each of the three enzymes achieved higher islet yields than Liberase HI. Although significant progress has been made in characterization of enzymes, further studies are necessary to determine the optimal enzyme blend.

Islet purification

In an earlier report, islets of rats purified from discontinuous sucrose gradients were not so sophisticated for *in vitro* metabolic studies of insulin biosynthesis (*2*). Thus, Lindall *et al.* (*39*) replaced the unpolymerized sucrose with layered isometric Ficoll, a polymer of sucrose, as the separating agent, and Scharp *et al.* (*40*) determined that rat islets obtained using dialyzed Ficoll exhibited improved insulin secretion. Meanwhile, Buitrago *et al.* (*41*) reported the rapid isolation of human and mouse pancreatic islets from collagenase-digested pancreas by sedimentation through a nontoxic low-osmotic density gradient medium, Percoll, which consists of colloidal silica particles coated with a layer of polyvinylpyrrolidone. A decade later, Scharp *et al.* (*42*) demonstrated that the Ficoll technique was much more effective for purifying human islets, providing about twice the yield of islets and insulin compared with the Percoll method. In 2000, the Edmonton protocol also adopted a Ficoll-based density gradient purification method with top loading using a semiautomated computerized COBE-2991 cell processor (*10*). Due to the suggestion that Ficoll is an islet toxin, Hering *et al.* investigated transplantation from a single donor using an iodixanol (OptiPrep^{TM})-based density gradient purification method and succeeded in achieving insulin independence (*11,43*). Later, Mita *et al.* (*44*) showed that the OptiPrep method significantly reduced the production of cytokines, including IL-1β, TNF-α, IFN-γ, IL-6, IL-8, MIP-1β, MCP-1, and RANTES, and improved β cell survival during pretransplant culture compared with the Ficoll purification method. In addition, Shimoda *et al.* developed a new purification method using top loading large bottles to reduce the shear stress seen with the COBE 2991 cell processor, thereby improving the quality and quantity of porcine and human islets after purification (*45,46*).

Instant blood-mediated inflammatory reaction

Despite considerable progress in the preparation of islets for transplantation, about 50% of transplanted islet cells are not detected in the liver in PET-CT images immediately after intraportal infusion of radio-labeled islets (*47*). In an *in vitro* model and pig-to-pig islet allotransplantation, Bennet *et al.* (*48*) observed macroscopic clotting within 5 min after the introduction of human islets into the blood, followed by continuous fibrin formation that generated a capsule surrounding the islets, complement activation, and infiltration with CD11+ leukocytes. They named this series of reactions 'instant blood-mediated inflammatory reaction' (IBMIR) and it could be prevented by a high heparin concentration in combination with the recombinant form of the complement inhibitor soluble complement receptor 1 (sCR1). Although it was recognized that IBMIR has a significant impact on the survival of transplanted islets, it is not easy to suppress IBMIR clinically. The addition of heparin is the current standard approach but it is insufficient.

Moberg *et al.* (*49*) determined that tissue factor (TF) from pancreatic islets serves as the main trigger of IBMIR and that the clotting reaction triggered by islets *in vitro* could be abrogated by blocking the active site of TF with specific antibodies or site-inactivated factor VIIa, a candidate drug for inhibition of TF activity. They also identified that the antioxidant nicotinamide inhibits TF and macrophage chemoattractant protein (MCP)-1 expression in isolated human pancreatic islets in an *in vitro* loop model (*50*). Their colleagues, Johansson *et al.* (*51*), revealed a negative correlation between thrombin-antithrombin complex generation 15 min after the first infusion of islets into the portal vein and fasting C-peptide production 7 days after transplantation.

Although Johansson *et al.* (*52*) reported in another study that low molecular weight dextran sulfate (LMW-DS; MW 5000) dose-dependently inhibited IBMIR by blocking coagulation and complement activation, a recent phase II, multicenter, open-label, active control, randomized study within the Clinical Islet Transplantation Consortium 01 study revealed that systemic LMW-DS treatment showed similar efficacy in preventing IBMIR and promoted islet engraftment to "state-of-the-art" treatment with heparin (*53*), leading to a reluctance to use LMW-DS because its target-activated partial thromboplastin time is 150 ± 10 s, which is 3 times longer than the 50 ± 5 s of heparin. This aspect is particularly important when performing total pancreatectomy followed by autologous islet transplantation. Notably, IBMIR also occurs in patients undergoing total pancreatectomy followed by autologous islet transplantation (*54*).

Islet surface engineering is another way to inhibit IBMIR. Cabric *et al.* (*55*) reported that heparin-coated islets protected against IBMIR in an allogeneic porcine model of islet transplantation. In addition to heparin-coating, other approaches reported to show protective effects against IBMIR include surface modification with polyethylene glycol (PEG)-lipid (*56*), surface modification with PEG-urokinase (*56*), co-immobilization of urokinase and the soluble domain of thrombomodulin (*57*), and immobilization of human soluble complement receptor 1 through PEG-conjugated phospholipid (PEG-lipid) (*58*). Further studies are thus required for the clinical application of islet transplantation.

Immunosuppression

The development of immunosuppressive agents has contributed greatly to transplant efficacy. Various immunosuppressive agents are listed in Table 1, and their applications in major clinical islet transplantation are shown in Table 2.

Early days of immunosuppression

Steroidal hormones, first extracted by Edward C. Kendall in 1934, have been used as anti-inflammatory agents for various inflammatory and autoimmune diseases since the successful use of cortisone to treat rheumatoid arthritis in 1948 by rheumatologist Phillip S. Hench (*59*). Meanwhile, Elion *et al.* (*60*) described the growth-inhibitory effect of 6-mercaptopurine (6-MP) on *Lactobacillus casei* in 1953 and Schwartz *et al.* (*61*) reported its suppressive effect on the formation of humoral antibody when given simultaneously with the antigen in 1958. Azathioprine (also called BW 57-322), a prodrug of 6-MP, was subsequently synthesized by George H. Hitchings and Gertrude Elion in 1957 (*62*). In contrast, cyclosporine (cyclosporin A), one of the most successful antirejection drugs to date, was obtained from the fermentation broth of the fungi *Trichoderma polysporum* and *Cylindrocarpon lucidum* in 1970 by Borel and Stahelin in an attempt to develop a new antifungal treatment. Its immunosuppressive properties were identified in 1971 and were accompanied by only weak myelotoxicity, a possible adverse effect of azathioprine (*63*).

Classical immunosuppressants in organ transplantation

The era of transplant therapy began with kidney transplantation. Because the first kidney transplantation between genetically unrelated individuals was performed in 1962 using prednisone and azathioprine for immunosuppression (*64*), this combination of glucocorticoid and azathioprine became the standard antirejection regimen. In 1966, Starzl *et al.* (*65*) used a horse antilymphocyte globulin (ALG) preparation in patients receiving a kidney transplantation as an adjunct to the standard immunosuppressive drugs, azathioprine and prednisone, whose doses were reduced in half the patients. The results of this study meant that this T cell-depleting polyclonal antibody, ALG, became one of the standard agents for the induction therapy, before being supplanted in the early 1980s by antithymocyte globulin (ATG) to avoid variations between lots of ALG (*66*). Moreover, since cyclosporine was introduced to clinical organ transplantation by Calne *et al.* in 1978 (*67*), the use of steroids was successfully reduced or avoided in cadaveric renal transplantation (*68*). Meanwhile, the use of high-dose intravenous methylprednisolone in renal transplantation was established; this strategy was associated with a very rapid lympholytic effect, a low incidence of complications, and the avoidance of tapering steroid doses (*69*).

Expansion of immunosuppression in islet transplantation

In the context of the immunosuppressive drugs used in organ transplantation, the first case of insulin independence after islet transplantation into the portal vein from cadaveric pancreases was achieved in 1990 in a type 1 diabetic patient premedicated with methylprednisolone 1 mg/kg and azathioprine 1 mg/kg and given Minnesota antilymphoblast globulin 20 mg/kg for 7 days starting 1 day after transplantation in addition to the stable cyclosporine dosage of 500 mg once daily for the existing kidney transplant (*9*).

A decade later, in 2000, three new immunosuppressive drugs (daclizumab, tacrolimus, and sirolimus) enabled 7 consecutive type 1 diabetes patients to achieve insulin independence over a median follow-up of 11.9 months after transplantation from 2 to 4 donor islets, without the use of the diabetogenic immunosuppressive drug, glucocorticoids, in the Edmonton protocol (*10*). Daclizumab, like basiliximab, is a monoclonal antibody

Table 1. List of immunosuppressants

Generic name	Effects	*Ref.*
Glucocorticoids	Genomic actions mediated through the classic glucocorticoid receptor (GR), a member of the nuclear receptor family of ligand-activated transcription factors, and rapid non-genomic actions *via* cytosolic GR or membrane-bound GR.	(*98*)
High-dose methylprednisolone	Rapid non-genomic actions mediated *via* cytosolic GR or membrane-bound GR.	(*69,98*)
Antilymphocyte globulin (ALG)	Polyclonal antibodies generated in animals by inoculation with human lymphoid cells.	(*66*)
Antithymocyte globulin (ATG)	Polyclonal antibodies generated in animals by inoculation with human thymocytes.	(*66*)
Azathioprine	A long-lived prodrug of 6-mercaptopurine (6-MP).	(*62*)
6-mercaptopurine (6-MP)	A purine antagonist.	(*60*)
Mycophenolate mofetil (MMF)	A relatively selective inhibitor of T and B cell proliferation; it is a prodrug of mycophenolic acid (MPA) synthesized to improve oral bioavailability.	(*74,78*)
Mycophenolic acid (MPA)	A 5-fold more potent inhibitor of the type II isoform of inosine-5′-monophosphate dehydrogenase (IMPDH), which is expressed in activated T and B lymphocytes, compared with the housekeeping type I isoform of IMPDH, which is expressed in most cell types, resulting in inhibition of T and B cell proliferation.	(*74,78*)
Cyclosporine (cyclosporin A, CsA)	Binds preferentially to cytosolic cyclophilin A, the predominant cyclophilin found within T cells, inhibiting calcineurin, a calcium/calmodulin-dependent serine threonine phosphatase. Inhibition of the dephosphorylation of the nuclear factor of activated T cells (NFAT) inhibits NFAT translocation from the cytoplasm to the nucleus, resulting in decreased transcription of interleukins (IL-2, IL-3, IL-4), TNF-alpha, and interferon-gamma (IFN-γ).	(*72,74*)
Tacrolimus (FK506)	Binds to FK506-binding proteins, particularly FKBP12, provoking inhibition of calcineurin, a phosphatase also inhibited by CsA.	(*72,73*)
Sirolimus (rapamycin)	Binds to FKBP12 to form a complex that binds to and inhibits the serine/threonine kinase mammalian target of rapamycin (mTOR), resulting in cell cycle arrest, repression of tumor cell growth, and inhibition of T and B cell proliferation.	(*74,76*)
Everolimus	A derivative of sirolimus.	(*76*)
Muromonab-CD3 (OKT3)	A mouse monoclonal antibody to CD3 primarily expressed on T cells that binds to CD3, thereby depleting T cells from the circulation and induces transient cytokine release syndrome.	(*70*)
hOKT3γ1(Ala-Ala)	A human IgG1-based OKT3 with amino acids 234 and 235 of the Fc region changed to alanine; this preserves the immunosuppressive effects of OKT3 but reduces the inductive effect on cytokine release syndrome.	(*77*)
Daclizumab	A monoclonal antibody targeting CD25, the alpha subunit of the IL-2 receptor that is expressed on activated T lymphocytes, leading to inhibition of T cell proliferation.	(*70*)
Basiliximab	Another monoclonal antibody targeting CD25, which inhibits T cell proliferation.	(*70*)
Etanercept	A monoclonal antibody to TNF-alpha that is particularly toxic to β cells.	(*99*)
Anakinra	An IL-1 receptor antagonist that promotes the survival of transplanted human islets; it has an additive effect with etanercept.	(*99*)
Alemtuzumab	A humanized, recombinant antibody to CD52, a cell surface glycosylphosphatidylinositol (GPI)-anchored glycoprotein expressed on T and B lymphocytes, monocytes, macrophages, and natural killer cells.	(*74,100*)
Efalizumab	A monoclonal antibody to CD11a, the alpha subunit of LFA-1, that inhibits the binding of intercellular adhesion molecule 1 (ICAM-1) to LFA-1, thereby suppressing the activities of LFA-1 expressed on all leukocytes. Efalizumab was withdrawn in 2009 due to concerns about the development of progressive multifocal myeloencephalopathy.	(*83*)
Belatacept	A fusion protein composed of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA-4 (cytotoxic T lymphocyte-associated protein 4), also known as CD152, which binds the ligands CD80 and CD86 on antigen-presenting cells, thereby inhibiting T cell activation.	(*83*)

targeting CD25 – the alpha subunit of the IL-2 receptor, which is expressed on activated T lymphocytes – and has fewer adverse effects compared with other monoclonal antibodies, such as the cytokine release syndrome caused by muromonab-CD3, a mouse monoclonal antibody against CD3 (trade name: Orthoclone OKT3) (*70*). Tacrolimus, also known as FK-506, is a macrolide compound derived from the fermentation broth of the fungus *Streptomyces tsukubaensis*, which was isolated from a soil sample collected near Mount Tsukuba in Japan by Fujisawa Pharmaceutical Company in 1984 (*71*). Although tacrolimus, as well as cyclosporine, is a calcineurin inhibitor that blocks dephosphorylation of the transcription factor nuclear factor of activated T cells, reducing the activity of genes encoding IL-2 and other related cytokines, it has been shown to be superior to cyclosporine in terms of graft survival and acute rejection (*72*). However, a recent report described detrimental effects of tacrolimus on β cells (*73*). Sirolimus, also called rapamycin, is another macrolide compound isolated from a fungus (*S. hygroscopicus*) from soil collected in the Vai Atoreregion on Easter Island (Rapa Nui) between December 1964 and February 1965; its immunosuppressive effect attracted attention in the 1980s after its structural homology with tacrolimus was noted, leading to its clinical use in organ transplantation in 1999 (*74*). The use of sirolimus enabled the glucocorticoid-free Edmonton immunosuppression protocol (*10*). However,

Table 2. List of major clinical islet transplantation trials

Year of publication (*Ref.*)	Induction	Maintenance	Insulin independence
1990 (*9*)	Cyclosporin, ALG, methylprednisolone, and azathioprine.	Cyclosporin and azathioprine.	1 patient achieved insulin independence at day 10-22.
2000 (*10*)	Daclizumab, sirolimus, and low-dose tacrolimus.	Sirolimus and low-dose tacrolimus.	All recipients (7 of 7) achieved insulin independence over a median follow-up of 11.9 months with 2 to 4 donor islets.
2004 (*20*)	hOKT3γ1(Ala-Ala) and sirolimus.	Sirolimus and low-dose tacrolimus.	Mean time to insulin independence was 35 ± 7 days in 4 of 6 patients with islets from a single-donor pancreas.
2005 (*11*)	ATG, methylprednisolone, daclizumab, and etanercept.	Sirolimus and reduced-dose tacrolimus. Tacrolimus was gradually replaced by MMF at 1 month posttransplantation.	5 of 8 recipients remained insulin-independent for longer than 1 year with only 1 islet infusion.
2008 (*79*)	ATG, methylprednisolone, and etanercept. Basiliximab instead of ATG and methylprednisolone before the second islet infusion.	Cyclosporine and everolimus. If deemed clinically appropriate, everolimus was replaced with MMF.	Of 6 recipients transplanted from 1 or 2 donor islets, 5 (83%) achieved insulin independence at 1 year and 4 continued to be insulin-independent at a mean of 3.4 ± 0.4 years.
2010 (*83*)	ATG and methylprednisolone before the first islet transplant and basiliximab before the second islet transplant.	Efalizumab or belatacept, followed by additional sirolimus and MMF. Tacrolimus and MMF.	All recipients (5 of 5) achieved insulin dependence over 1 year with 1 or 2 islet transplants.
2013 (*80*)	ATG, tacrolimus, and MMF. Basiliximab instead of ATG for the third islet transplant.	Tacrolimus converted to sirolimus 6 months after the final transplant, if tolerated.	Insulin independence was achieved 1 year after the first islet infusion with 1 to 3 islet infusions in 52.9% (7 of 19 recipients).
2016 (*12*)	ATG and etanercept. Basiliximab replacing ATG at subsequent transplant.	Sirolimus and low-dose tacrolimus.	Insulin independence was achieved at day 365 in 52.1% (25 of 48 recipients). About half of the recipients received 1 islet infusion.
2020 (*82*)	Anakinra, etanercept, and ATG (first 7 patients) or alemtuzumab (next 2 patients). Steroids for premedication before ATG.	Tacrolimus and MMF.	Insulin independence was achieved at 1 year with 1 or 2 islet infusions in 44% (4 of 9 recipients).
2020 (*87*)	Reparixin or placebo along with ATG or basiliximab (plus bolus of methylprednisolone prior to ATG) before the first islet infusion and basiliximab before the second islet infusion.	A cell proliferation inhibitor (either sirolimus or MMF) and a calcineurin inhibitor (preferentially tacrolimus, but cyclosporine could also be used).	Insulin independence was achieved 1 year after the last islet infusion with 1 or 2 islet infusions in 32.0% (8 of 25 recipients) in the reparixin group vs. 31.3% (5 of 16 recipients) in the placebo group.

the rate of insulin independence at 2 years was only 14% (*75*), partly because sirolimus has detrimental effects on pancreatic β cells (*76*).

After establishment of the Edmonton protocol

To improve long-term outcomes in clinical islet transplantation, various new immunosuppressants have been developed and used. Although muromonab-CD3, a mouse monoclonal antibody to CD3 (Orthoclone OKT3), is effective for reversing renal transplant rejection episodes, it can induce cytokine release syndrome, which is characterized by flu-like symptoms and, occasionally, severe hypotension, bronchospasm, tachycardia, and even death (*70*). To reduce this human anti-mouse antibody response, Xu *et al.* (*77*) constructed hOKT3γ1(Ala-Ala), a human IgG1-based OKT3 with amino acids 234 and 235 of the Fc region changed to alanine, which prevents binding to the Fc receptor. Hering *et al.* (*20*) applied hOKT3γ1(Ala-Ala) along with sirolimus for induction immunosuppression to allogeneic islet transplant, followed by sirolimus and low-dose tacrolimus for maintenance immunosuppression. This protocol enabled 4 of 6 patients with type 1 diabetes to achieve insulin independence at a mean time of 35 ± 7 days after transplantation.

However, Hering *et al.* had previously reported in 2005 (*11*) that the first single-donor, marginal-dose islet transplantation restored insulin independence in all 8 treated patients with type 1 diabetes. This protocol comprised rabbit antithymocyte globulin (rATG), methylprednisolone, daclizumab, and etanercept for

induction immunosuppression and sirolimus and reduced-dose tacrolimus for maintenance immunosuppression, with tacrolimus gradually replaced by mycophenolate mofetil (MMF) at 1-month posttransplantation. The results suggest the importance of inhibiting tumor necrosis factor with etanercept in the peritransplant period and replacing tacrolimus. Furthermore, 5 of the 8 recipients remained insulin-independent for longer than 1 year.

MMF is a prodrug of mycophenolic acid (MPA), which was first discovered as a fermentation product of *Penicillium brevicompactum* and related fungi in 1893 (*74,78*). Because MPA was found to be a 5-fold more potent inhibitor of the type II isoform of inosine-5′-monophosphate dehydrogenase (IMPDH), which is expressed in activated T and B lymphocytes, than of the housekeeping type I isoform of IMPDH, which is expressed in most cell types, MMF was developed as an effective immunosuppressive prodrug of MPA that preferentially inhibits the proliferation of human T and B lymphocytes.

A similar immunosuppression protocol using rATG and etanercept for induction and sirolimus and low-dose tacrolimus for maintenance was adopted in a phase 3 trial of human islet transplantation for type 1 diabetes complicated by severe hypoglycemia (*12*). Insulin independence was achieved in 52.1% of the patients at day 365, about half of whom received only 1 islet infusion. Moreover, the same group from the University of Minnesota reported in 2008 that, of 6 patients who received transplants from 1 or 2 donor islets, 5 achieved insulin independence at 1 year and 4 continued to be insulin-independent at a mean of 3.4 ± 0.4 years posttransplant (*79*). Their protocol used rATG, methylprednisolone, basiliximab, and etanercept for induction immunosuppression and cyclosporine and everolimus, a derivative of sirolimus, for maintenance immunosuppression. If deemed clinically appropriate, everolimus was replaced with MMF, suggesting that it is important to avoid tacrolimus and sirolimus, both of which have diabetogenic effects (*72,76*).

In contrast, a multicenter Australian trial (*80*) of islet transplantation that used rATG, tacrolimus, and MMF for induction and tacrolimus and MMF for maintenance with conversion of tacrolimus to sirolimus 6 months after the final transplant if tolerated achieved insulin independence in 52.9% of patients at 1 year after the first islet infusion, despite the use of 1 to 3 islet infusions, implying the beneficial effect of the addition of an anti-TNF agent, as used in the previous report from the University of Minnesota group. On the other hand, Matsumoto *et al.* (*81*) reported in 2011 the favorable effects of the IL-1 receptor antagonist anakinra in combination with etanercept for induction. This was followed by a recent phase 1/2 clinical trial (ClinicalTrials.gov, NCT00530686) (*82*). Although double blockade of TNF-α and IL-1 by etanercept and anakinra in the peritransplantation period was shown to be safe in this recent trial, only 4 of the 9 patients achieved insulin independence with 1 or 2 islet infusions at 24 months after the transplantation, suggesting that other strategies are required to maintain long-term islet function.

Distinct from the above-mentioned clinical studies, Posselt *et al.* (*83*) of the University of California reported calcineurin inhibitor-free immunosuppressive regimens using efalizumab, an anti-leukocyte functional antigen-1 (anti-LFA-1) antibody, or belatacept, a fusion protein composed of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA-4 (cytotoxic T lymphocyte-associated protein 4; also known as CD152), which achieved insulin independence for over 1 year with 1 or 2 islet transplants. The immunosuppressants were rATG and methylprednisolone for induction and efalizumab or belatacept for maintenance, followed by additional sirolimus, and MMF. The results demonstrated the considerable benefits of efalizumab and belatacept for both the engraftment and survival of transplanted islets.

Efalizumab is a humanized monoclonal antibody against CD11a, the alpha subunit of LFA-1, that inhibits the ability of intercellular adhesion molecule 1 (ICAM-1) to bind to LFA-1, thereby suppressing the activities of LFA-1 expressed on all leukocytes. However, efalizumab was withdrawn from clinical use in 2009 due to concerns about the development of progressive multifocal myeloencephalopathy caused by reactivation of latent JC virus infection (*83*).

Belatacept, a fusion protein composed of the Fc fragment of human IgG1 linked to the extracellular domain of CTLA-4, binds to the ligands CD80 and CD86 on antigen-presenting cells, thereby inhibiting T cell activation. Because this drug has been successfully used in a phase 3 kidney transplantation study (*84*), it would also be an attractive immunosuppressive agent for pancreatic islet transplantation. Moreover, in the above-mentioned multicenter Australian trial, 1 recipient remained insulin-independent after switching to belatacept and MMF 6 months after the final islet transplantation because the patient could not change from MMF and tacrolimus to MMF and sirolimus due to severe anemia and edema (*80*). As discussed by Barlow *et al.* (*76*), the reason MMF is increasingly used instead of sirolimus in clinical islet programs, despite the detrimental effect of MMF on glucose-stimulated insulin secretion (GSIS) in human islets, may be the adverse effects of sirolimus rather than its toxicity to β cells.

Because the CXCL1–CXCR1/2 axis is another therapeutic target of inflammatory cytokines (*85*), reparixin, an inhibitor of CXC chemokine receptor types 1 (CXCR1) and 2 (CXCR2), was used to enhance pancreatic islet survival after transplantation in a phase 2, randomized, open-label, pilot study involving a single infusion of allogeneic islets (*86*). However, a recent

phase 3, multicenter, randomized, double-blind, parallel-assignment study (ClinicalTrials.gov, NCT01817959) showed no significant effects of reparixin on stimulated C-peptide production during the mixed-meal tolerance test at 75 ± 5 days after the first transplant and 365 ± 14 days after the last transplant, on the rate of early insulin independence or at 1 year after islet transplantation, or on any other secondary measures of glycemic control (*87*). Although the results were disappointing, there was a higher tendency for recipients in the reparixin group to achieve insulin independence after the first islet infusion, suggesting that the combination of ATG and reparixin was favorable. Further studies using new immunosuppressive agents and their combination with conventional immunosuppressants hold promise for achieving more efficient islet transplantation.

Islet transplantation in Japan

The clinical islet transplantation program in Japan has been run by the Japan Society for Pancreas and Islet Transplantation since 1997 (*88*). Following the first islet transplantation in Japan conducted by a Kyoto University group in 2004 (*31*), 18 patients with type 1 diabetes had received isolated islets from 33 non-heart-beating donors by 2007. All patients showed functioning islets, and 3 patients who received multiple transplants achieved temporary insulin independence (*89*). However, most recipients failed to maintain detectable C-peptide levels 5 years after transplantation. In April 2007, islet transplantation was suspended because the collagenase used in the islet isolation was purified from culture supernatants of *Clostridium histiolyticum* grown in medium containing brain heart infusion broth, which is a potential source of transmissible bovine spongiform encephalopathy (*88*). After the development of a mammalian-free collagenase, a phase 2, multicenter, clinical trial of islet transplantation for type 1 diabetes patients (UMIN Clinical Trials Registry: UMIN000003977) was started in 2012 based on the protocol used in phase 3 trials in North America (*12*) is currently ongoing and will end soon. Because sirolimus has not been approved in Japan as an immunosuppressant for transplantation, MMF has been used from the beginning of the maintenance period. From 2013, brain-dead donors as well as non-heart-beating donors were approved as sources of transplantable islets. The success rate of the islet isolation was very high according to an interim report (*90*) and the outcomes were favorable, which led to allogeneic islet transplantation being covered by the national health insurance in Japan beginning in April 2020 (*https://www.mhlw.go.jp/content/12400000/000602944.pdf*).

Alternative islet sources

Because of the overwhelming shortage of allogeneic donor islets, alternative islet sources are desirable. Pigs could provide an alternative source of organs and cells, even after taking into consideration the potential risks of xenotransplantation and porcine endogenous retrovirus infection (*91,92*). The first transplantation of porcine fetal islet-like cell clusters into diabetes patients using immunosuppressants was reported in 1994 (*93*). Four of the 10 insulin-dependent diabetic kidney transplant patients excreted small amounts of porcine C-peptide in urine for 200-400 days but were unable to reduce their insulin injection dose. Meanwhile, transplantation of alginate-poly-L-ornithine-alginate (APA)-encapsulated neonatal porcine islets to 14 patients with unstable type 1 diabetes was performed in New Zealand from 2009 to 2011 without immunosuppressants. The results showed no detection of porcine endogenous retrovirus DNA and RNA, decreased frequency of unaware hypoglycemic events, and a slightly decreased dose of daily injected insulin in the patients transplanted with a lower number of islets, implying that too many transplanted islets compromised oxygen and nutrition supply (*94*). A modified protocol performed in Argentina successfully reduced HbA1c in all 8 patients, with a reduction in daily insulin dose in 5 of the patients (*95*).

Stem cell-derived pancreatic progenitors and insulin-producing cells are also promising sources of cells for β cell replacement therapies for type 1 diabetes. These cells are often combined with macroencapsulation, which provides immune isolation and eliminates the need for immunosuppressive drugs. Moreover, there is the option for them to be removed from the recipient in case of emergency, mainly because of tumorigenicity derived from undifferentiated embryonic or pluripotent stem cells that might be included in the transplanted cell clusters. A phase 1/2 clinical trial using human embryonic stem cell-derived pancreatic progenitors in a macroencapsulation device (VC-02 by ViaCyte, Inc.) is ongoing in the United States, having started in 2017 (ClinicalTrials.gov, NCT03163511). Although stem cell-derived pancreatic progenitors and insulin-producing cells are more resistant than mature islets to hypoxia and nutrient deprivation, long-term viability has not yet been achieved. Further studies are being conducted. Amino acid supplementation during transplantation and devices that can supply oxygen are promising avenues of research (*96,97*).

Conclusion

Transplantation of pancreatic islets from allogeneic donors has been widely performed. Although Japan was slow to establish islet transplantation as a standard treatment for type 1 diabetes, the national health insurance system in Japan has recently begun to cover allogeneic islet transplantation from deceased donors. The shortage of donor islets has yet to be solved, but several clinical trials using xenogeneic and stem cell-

derived sources are ongoing and their results are eagerly awaited. It is hoped that they will contribute to establishing alternative sources for insulin-producing β cells in the near future.

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REVIEW

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Is single acupoint Sanyinjiao (SP 6) effective in managing insomnia? A systematic review of randomized controlled trials

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Abstract: Insomnia is a symptom of physical or mental disorder and refers to decreased sleep time and potentially low quality. There is evidence showing that acupuncture could enhance management of insomnia, and Sanyinjiao (SP 6) is one of the promising acupoints. This review aimed to evaluate the effectiveness of stimulating single acupoint SP 6 in managing insomnia. The study was registered under PROSPERO CRD42019140855. English and Chinese databases were searched for randomized controlled trials on single acupoint SP 6 stimulation in management of insomnia. Quality of methodology was assessed by two authors independently using the Cochrane Risk of Bias Tool, and reporting quality was assessed by the STRICTA checklist. The primary outcome was the Pittsburgh Sleep Quality Index (PSQI), and secondary outcomes were improvement in clinical effect and sleep duration assessed by polysomnogram (PSG). The extracted data were pooled and meta-analyzed with RevMan 5.3 software. Four trials involving 288 participants were included in this review. The findings showed that SP 6 stimulation could improve sleep quality (MD -0.30, 95% CI [-0.52, -0.08]), lengthen deep sleep duration (MD 80.46, 95% CI [56.47, 104.45]), rapid eye movement (REM) duration (MD 91.53, 95% CI [68.41, 114.65]), and increase improvement in clinical effect. Quality of reporting and methodology was limited in all included trials. Some limited evidence showed that single acupoint stimulation of SP 6 could improve sleep quality, lengthen deep sleep and REM duration of patients with insomnia. However, the findings in this review should be interpreted with caution due to methodological limitations.

***Keywords*:** acupuncture, single point, Sanyinjiao, SP 6, insomnia, meta-analysis

Introduction

Good quality sleep is the foundation of healthy life as a normal person spends more than 30% of their whole life time in bed (*1*). More than 27% of people worldwide have problems with sleeping (*2*), among which 6%-10% meet the diagnostic criteria for primary insomnia (*3*). Insomnia is a condition of unsatisfactory sleep, in terms of sleep onset, and early waking. Insomnia is also a subjective condition, which can cause psychological discomfort including stress or mood disturbances because of low quality sleep and lack of sleeping time. Patients with insomnia may complain about difficulty falling asleep and/or early waking (*4*). Meanwhile, insomnia can be secondary to other diseases including anxiety and depression and is linked to increased incidence of cardiovascular diseases (*5,6*). A study reported that there are relationships between insomnia and paranoia, which has received sustained attention (*7*). In the United Kingdom (UK), the prevalence of primary insomnia is increasing (between 3.1% and 5.8%) and it is 1.5-2 times higher in women than in men (*8*). In addition to the above, insomnia is mostly considered a long-term dysfunction. A large UK study reported that approximate 75% of insomnia patients had symptoms lasting at least one year (*9*), and a three-year longitudinal study suggested that about 46% reported insomnia lasting three years (*10*).

Hypnotics have been reported to have good efficacy for managing insomnia, but there is still lack of evidence to prove hypnotics are the best choice to manage insomnia because of the significant adverse effects including daytime sedation, poor motor coordination, related concerns about driving accidents and injuries from falls, addictiveness and the effect on cognitive function (*11-13*). Some other adverse effects have also been reported: a fourteen-year follow-up study conducted in Taiwan showed that hypnotics increased the risks of oral and breast cancer in insomniacs with a hazard ratio of 1.49 times compared with insomnia patients

who did not use hypnotics (*14*). A study performed in Korea showed that there were some associations between hypnotics and mortality (*15*). On the other hand, tolerance to hypnotics is another cause reducing their effectiveness for managing insomnia (*16*).

Traditional Chinese Medicine (TCM), especially acupuncture, has been widely accepted in many regions for some specific diseases. Sleep disorders are one of the preponderant illnesses for acupuncture therapy (*17*). Research evidence from a systematic review including 5,533 participants with all types of insomnia suggested that acupuncture reduced Pittsburgh Sleep Quality Index (PSQI) scores and appeared to be safe in comparison to Western medications (*18*). After a one-year follow-up with a 40-year-old insomnia patient, Zhang *et al.* reported that acupuncture improved sleep quality and accompaning symptoms (morning headache, fatigue, and mood worsening) by polysomnographic readings and therefore it may be considered to be used as primary and independent treatment for chronic insomnia (*19*). A Bayesian network meta-analysis of 42 studies involving 3,304 participants confirmed that scalp acupuncture might be the most effective intervention for primary insomnia compared with Western medicine, electro-acupuncture, warm acupuncture, and conventional acupuncture measured by PSQI score (*20*). In TCM theory, the cause of insomnia is an imbalance of *Yang* [阳] and *Yin* [阴], therefore the treatment of insomnia is to regulate the balance of *Yang* and *Yin*. Lower and inner parts of the body indicate *Yin* in TCM, and the sixth point of the Spleen meridian, Shanyinjiao (SP 6), is an acupoint where three *Yin* meridians (spleen, liver and kidney meridians, which run from the foot to the head) and converge near the inner ankle, so stimulation of SP 6 could activate the three *Yin* meridians and used as an optimal point for balancing *Yin* in the whole body. SP 6 is known to have an association with sleep because of its location and efficacy on coordinating *Yang* and *Yin* in the body. It records that SP 6 could calm the heart and tranquilize the mind from Classic Acupuncture and Moxibustion (known as Zhenjiu Jiayi Jing in Chinese). In clinical practice for insomnia, SP 6 is the second most commonly used acupoint after Shenmen (HT 7), at a frequency around 67% (*21*). Animal-based research showed that SP 6 stimulation improves the condition of sleep disorders maybe through increasing the content of 5-hydroxytryptamine (5-HT) in the hippocampus (*22*) or regulating energy metabolism in the paraventricular nucleus of the hypothalamus (*23*).

It will take a lot of work to make an easy-to-administer protocol on acupoint selection for managing insomnia because of the issue around blinding and lack of standardization in point selection, but we aim to offer a list of possible acupoints for managing insomnia by analyzing the effect of single acupoints individually. In our previous research, we showed that Shenmen (HT 7) could be a possible option for managing insomnia effectively (*24*). In this systematic review, the effectiveness of stimulating SP 6 for managing insomnia will be evaluated as a standardizable single point.

Materials and Methods

This study was reported following the Preferred Reporting Item for Systematic Review and Meta-Analysis (PRISMA) (*25*), and has been registered under PROSPERO (registration number CRD42019140855) (*26*).

Data sources and search items

Databases include PubMed, ScienceDirect, Web of Science, Cochrane Library, China National Knowledge Infrastructure (CNKI), Wanfang and Chinese Science and Technology Journal Database (VIP). Databases were searched from their inceptions to April 2019. The search strategy was developed following the guidelines of Cochrane Review Handbook. The search items are shown in Supplement Table S1 (*https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=5*).

Inclusion criteria

Types of studies: Randomized controlled trials (RCTs) which evaluated the effectiveness of single acupoint Sanyinjiao (SP 6) for managing insomnia were included in this review, regardless of blinding, and publication region.

Types of participants: Participants diagnosed with clinical insomnia were included in this review, irrespective of age, gender, cause of insomnia, or the duration of insomnia.

Types of interventions: The interventions of treatment group were any stimulation at SP 6, including acupuncture, acupressure, moxibustion, catgut-embedding, acupoint application, acupoint injection, or other relevant stimulations. The comparisons included no treatment, hypnotics, sham, pseudo points (points nearby acupoints) stimulation, Chinese herbs, or other possible treatments.

Types of outcome measures: The primary outcome was Pittsburgh Sleep Quality Index (PSQI, score from 0 to 21, and lower scores indicate improvement in sleep), which includes seven items: global scores, quality of sleep, sleep latency, sleep duration, sleep efficiency, sleep disturbance, and daytime function. Higher PSQI scores indicated worse sleep.

The secondary outcomes were improvement in clinical effect and sleep duration monitoring by polysomnogram (PSG). PSG included four items: latency to persistent sleep, light sleep duration, deep sleep duration, and rapid eye movement (REM) duration. The higher scores of the first two items indicated worse sleep, while higher scores of the last two items indicated

better sleep. All the outcomes were analyzed by using the values after treatment and at the follow-up points (if possible).

Data extraction

Data of study characteristics extracted from the included trials included study ID, sample size, age of participants, stimulation on SP 6, duration of stimulation, stimulating side of SP 6, interventions of control groups, outcomes, and their follow-ups.

Risk of bias and reporting quality of included trials

Two authors (ZJW, YZ) assessed risk of bias, reporting and methodology quality according to Cochrane Review Handbook by using Review Manager 5.3 software (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) independently. Seven items were judged as low, high or unclear risk of bias. Different opinions were discussed by the two authors, and the third author (XYH) made the decision if discussions did not reach a consensus.

Reporting quality of the included trials was assessed by STRICTA checklist (*27*). Full, partial and no reporting were judged for each item. High reporting quality was defined as more than 70% of items being fully reported, whereas 50-70% indicated medium quality, 30-50% indicated low quality, and ≤ 30% indicated very low quality (*27*).

Data synthesis and analysis

Data was synthesized and analyzed using Review Manager 5.3 software. For dichotomous data we calculated the risk ratio (RR) with 95% confidence interval (CI) using the Mantel-Haenszel (M-H) method. For continuous data we calculated the mean difference (MD). The choice of effect model depended on the heterogeneity test: if $I^2 \geq 50\%$ and the p value of the Chi^2 was less than 0.1, we chose the random-effects model because of high heterogeneity, otherwise we chose the fixed-effects model. The reasons for heterogeneity may be different types of stimulations in every trial and duration of stimulation, which would be sensitivity analyses if enough trials were included. Subgroup analysis would be conducted if possible according to different ages of participants, and data from each subgroup would be evaluated independently. If there were less than three trials in an analysis group that meta-analysis would be impossible, in which case a narrative description would be performed.

Results

An initial search identified 1,081 potential studies, and 586 were excluded by removing duplicates. The remaining 495 studies were scanned by title and abstract, with 487 studies excluded because of not meeting the inclusion criteria. The remaining eight trials were screened with full texts and four trials were finally included in this study (Figure 1).

Study characteristics

Four trials (*28-31*) with 288 participants were involved in this study. All the included trials were conducted and published in mainland China. The condition in three of them was primary insomnia (*28-30*), one was insomnia after surgery (*31*). The outcome in all four trials included PSQI, two (*29,30*) reported improvement in clinical effect of sleeping disorders as an outcome, and one (*31*) reported PSG. Three trials (*29-31*) had a four-week follow-up. The detailed study characteristics are shown in Table 1.

The stimulation on SP 6 in one trial (*28*) was warm-acupuncture (put a burning moxa stick on the needle handle), two (*29,30*) were acupuncture, and one (*30*) was acupoint herbal plaster. Three trials (*29-31*) reported that SP6 was stimulated on both sides.

Quality of reporting

The reporting quality of the four trials was low. According to the STRICTA checklist, two included trials (*29,30*) were judged low quality because the percentage of full reporting was 48.6% and 45.9% respectively, and the other two trials (*28,31*) were very low quality with 29.7% of the items being fully reported (Table 2).

Risk of bias

Quality of methodology was limited in the four trials (Figure 2). Regarding randomization, one trial (*29*) reported using random number cards for random sequence generation, so low risk was judged, one (*28*) failed randomization by grouping participants according to the treatment plans, so high risk was judged, the other two (*30,31*) reported randomization without methods, so unclear risk was judged. Two included trials (*29,30*) reported using sealed envelopes, which were judged low risk. One trial (*30*) reported blinding of outcomes assessment. Two (*29,30*) did not fully report outcome data, so high risk of bias was judged. Two trials (*29,30*) reported similarity of baseline characteristics, detailed information of criteria, ethical approval, and funding, so low risk of other bias was judged.

Effect estimation

Pittsburgh Sleep Quality Index (PSQI): All included trials reported PSQI as an outcome, but two trials (*29,30*) did not report the exact values of PSQI after treatment, so only two trials (*28,31*) were evaluated, which showed

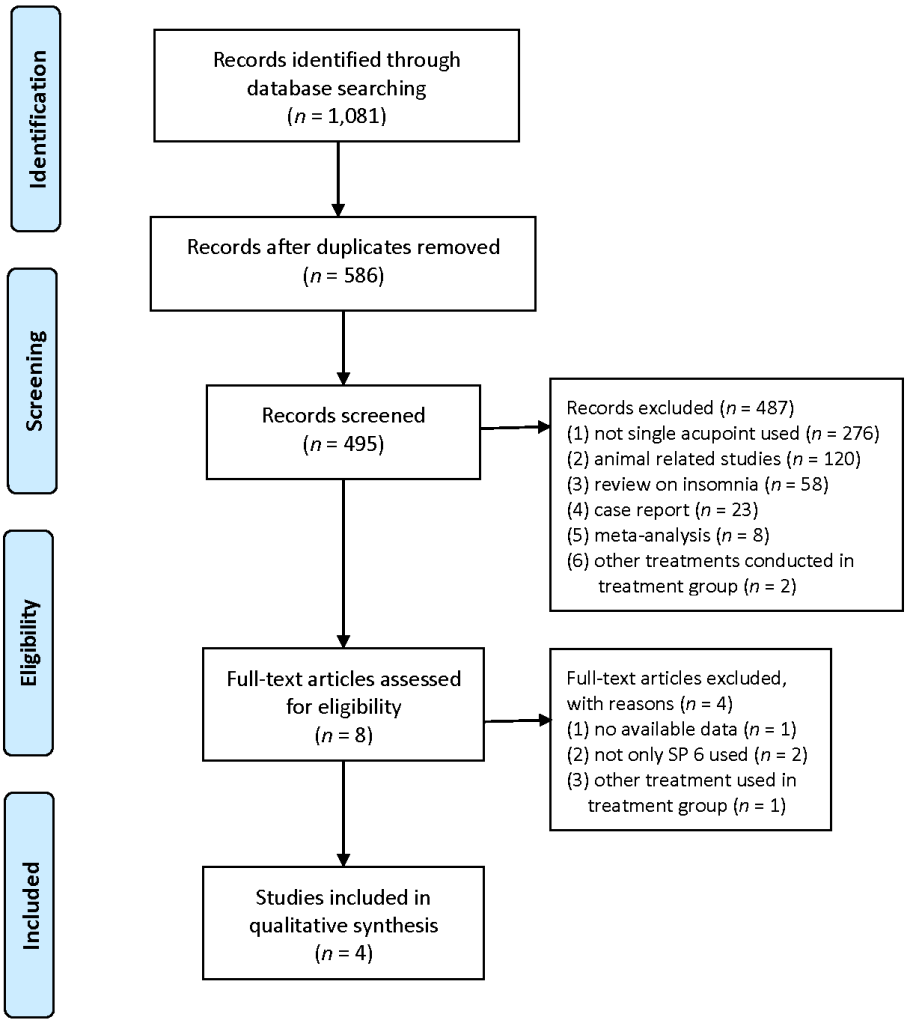

Figure1. Flow diagram.

Table 1. Study characteristics of the included trials

Study (*Ref.*)	Conditions	Sample size (T/C)	Age (T/C) (year)	Stimulation	Duration	Side of point	Outcomes	Follow-ups
Geng 2017 (*28*)	Primary insomnia	50/45	41.8/43.8	warm-acupuncture	50 min for 25 days	both sides	PSQI	NR
Zhao 2017 (*29*)	Primary insomnia	34/32	51.67 ± 8.7/ 50.96 ± 8.46	acupuncture	30 min for 25 days	both sides	PSQI, improvement in clinical effect	4-week
Shi 2017 (*30*)	Primary insomnia	33/34	48.64 ± 8.31/ 50.26 ± 6.43	acupuncture	30 min for 25 days	NR	PSQI, improvement in clinical effect	4-week
Li 2018 (*31*)	Insomnia after surgery	30/30	20-70	acupoint herbal plaster	20 min for 7 days	both sides	PSQI, PSG	4-week

C: control group; NR: not reported; PSG: polysomnogram; PSQI: Pittsburgh Sleep Quality Index; sham: replace the herbal content with saline in the plaster; T: treatment group.

that stimulations on SP 6 were more effective than moxa on Shenmen acupoint intervention or sham group in global score of PSQI (MD -2.77, 95% CI [-3.84, -1.71], I^2 = 97%, p < 0.00001). One trial (*28*) reported six aspects of PSQI separately showing that warm-acupuncture on SP 6 was more effective than moxa on Shenmen acupoint in improving quality of sleep (MD -0.30, 95% CI [-0.52, -0.08]), while there was no difference in sleep latency (MD -0.25, 95% CI [-0.59, 0.09]), sleep duration (MD -0.26, 95% CI [-0.82, 0.30]), sleep efficiency (MD -0.03, 95% CI [-0.53, 0.47]), sleep disturbance (MD -0.16, 95% CI [-0.42, 0.10]), or daytime function (MD -0.12, 95% CI [-0.29, 0.05]).

Improvement in clinical effect: Two included trials

Table 2. Reporting quality evaluation by STRICTA checklist

Section/Topic	No.	Item	Assessment (*Ref.*)			
			Geng 2017 (*28*)	Zhao 2017 (*29*)	Shi 2017 (*30*)	Li 2018 (*31*)
Title and abstract						
	1a	Identification as a randomized trial in the title	N	N	N	N
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	Y	Y	Y	Y
Introduction						
Background and objectives	2a	Scientific background and explanation of rationale	Y	Y	Y	Y
	2b	Specific objectives or hypotheses	Y	Y	Y	Y
Methods						
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	N	Y	Y	N
	3b	Important changes in methods after trial commencement (such as eligibility criteria), with reasons	N	N	N	N
Participants	4a	Eligibility criteria for participants	Y	Y	Y	Y
	4b	Settings and locations where the data were collected	Y	Y	Y	Y
Acupuncture rationale	5a	Style of acupuncture (*e.g.* Traditional Chinese Medicine, Japanese, Korean, Western medical, Five Element, ear acupuncture, *etc.*)	Y	Y	Y	Y
	5b	Reasoning for treatment provided, based on historical context, literature sources, and/or consensus methods, with references where appropriate	P	Y	P	P
	5c	Extent to which treatment was varied	Y	Y	Y	Y
Details of needling	6a	Number of needle insertions per subject per session (mean and range where relevant)	P	P	P	P
	6b	Names (or location if no standard name) of points used (uni/bilateral)	Y	Y	Y	Y
	6c	Depth of insertion, based on a specified unit of measurement, or on a particular tissue level	P	P	P	P
	6d	Response sought (*e.g.* de qi or muscle twitch response)	Y	Y	Y	Y
	6e	Needle stimulation (*e.g.* manual, electrical)	Y	Y	Y	Y
	6f	Needle retention time	Y	Y	Y	Y
	6g	Needle type (diameter, length, and manufacturer or material)	Y	Y	Y	Y
Treatment regimen	7a	Number of treatment sessions	Y	Y	Y	Y
	7b	Frequency and duration of treatment sessions	Y	Y	Y	Y
Other components of treatment	8a	Details of other interventions administered to the acupuncture group (*e.g.* moxibustion, cupping, herbs, exercises, lifestyle advice)	Y	Y	Y	Y
	8b	Setting and context of treatment, including instructions to practitioners, and information and explanations to patients	N	N	N	N
Practitioner background	9	Description of participating acupuncturists (qualification or professional affiliation, years in acupuncture practice, other relevant experience)	N	N	N	N
Control or comparator	10a	Rationale for the control or comparator in the context of the research question, with sources that justify this choice	P	P	P	P
	10b	Precise description of the control or comparator. If sham acupuncture or any other type of acupuncture-like control is used, provide details as for items 5 to 8 above	P	P	P	P
Outcomes	11a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	P	P	P	P
	11b	Any changes to trial outcomes after the trial commenced, with reasons	N	Y	Y	N
Sample size	12a	How sample size was determined	N	N	N	N
	12b	When applicable, explanation of any interim analyses and stopping guidelines	N	N	N	N
Randomization						
Sequence generation	13a	Method used to generate the random allocation sequence	N	Y	N	N
	13b	Type of randomization; details of any restriction (such as blocking and block size)	N	P	P	N
Allocation concealment mechanism	14	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	N	Y	N	N
Implementation	15	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	N	N	N	N

N: no reporting; P: partly reporting; Y: fully reporting. Evaluation criteria depended on the percentage of fully reporting items: ≤ 30%: Very Low quality (VL); 30-50%: Low quality (L); 50-70%: Medium quality (M); > 70%: High quality (H).

Table 2. Reporting quality evaluation by STRICTA checklist (continued)

Section/Topic	No.	Item	Assessment (*Ref.*)			
			Geng 2017 (*28*)	Zhao 2017 (*29*)	Shi 2017 (*30*)	Li 2018 (*31*)
Randomization						
Blinding	16a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N	N	N	N
	16b	If relevant, description of the similarity of interventions	N	Y	Y	N
Statistical methods	17a	Statistical methods used to compare groups for primary and secondary outcomes	Y	Y	Y	Y
	17b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	N	N	N	N
Results						
Participant flow (a diagram is strongly recommended)	18a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	N	P	P	N
	18b	For each group, losses and exclusions after randomization, together with reasons	Y	Y	Y	Y
Recruitment	19a	Dates defining the periods of recruitment and follow-up	N	Y	Y	Y
	19b	Why the trial ended or was stopped	N	N	N	N
Baseline data	20	A table showing baseline demographic and clinical characteristics for each group	N	Y	Y	N
Numbers analyzed	21	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Y	Y	Y	Y
Outcomes and estimation	22a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Y	Y	Y	Y
	22b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N	N	N	N
Ancillary analyses	23	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N	N	N	N
Harms	24	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N	P	P	N
Discussion						
Limitations	25	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	N	P	P	N
Generalizability	26	Generalizability (external validity, applicability) of the trial findings	Y	Y	Y	Y
Interpretation	27	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	P	P	P	P
Other information						
Registration	28	Registration number and name of trial registry	N	N	N	N
Protocol	29	Where the full trial protocol can be accessed, if available	N	N	N	N
Funding	30	Sources of funding and other support (such as supply of drugs), role of funders	Y	Y	Y	N
RATING OVERALL CONFIDENCE IN THE RESULITS OF THE TRIALS			VL	L	L	VL

N: no reporting; P: partly reporting; Y: fully reporting. Evaluation criteria depended on the percentage of fully reporting items: ≤ 30%: Very Low quality (VL); 30-50%: Low quality (L); 50-70%: Medium quality (M); > 70%: High quality (H).

(*29,30*) suggested that acupuncture on SP 6 improved clinical effects in comparison with pseudo acupoint/multi-acupoints stimulations (RR 0.76, 95% CI [0.60, 0.98]), but could improve clinical effects in comparison with pseudo acupoint (RR 1.52, 95% CI [0.93, 2.50]).

Sleep duration: One trial (*31*) assessed PSG by observing participants in sleep for 20 minutes every day, with 15 seconds as a measurement unit. It showed that acupoint herbal plaster on SP 6 improved sleep duration as measured by PSG when compared with a sham group, with shorter latency to persistent sleep (MD -32.35 seconds, 95% CI [-43.78, -20.92]), shorter light sleep duration in seconds (MD -29.70, 95% CI [-52.31, -7.09]), longer deep sleep duration in seconds (MD 80.46, 95% CI [56.47, 104.45]), and longer REM duration in seconds (MD 91.53, 95% CI [68.41, 114.65]).

Adverse events: Only one included trial (*29*) reported adverse events, showing that there was no adverse event in both acupuncture on SP 6 group or pseudo acupoint stimulation group during the whole 25-days of research.

Discussion

This is a systematic review focusing on single acupoint

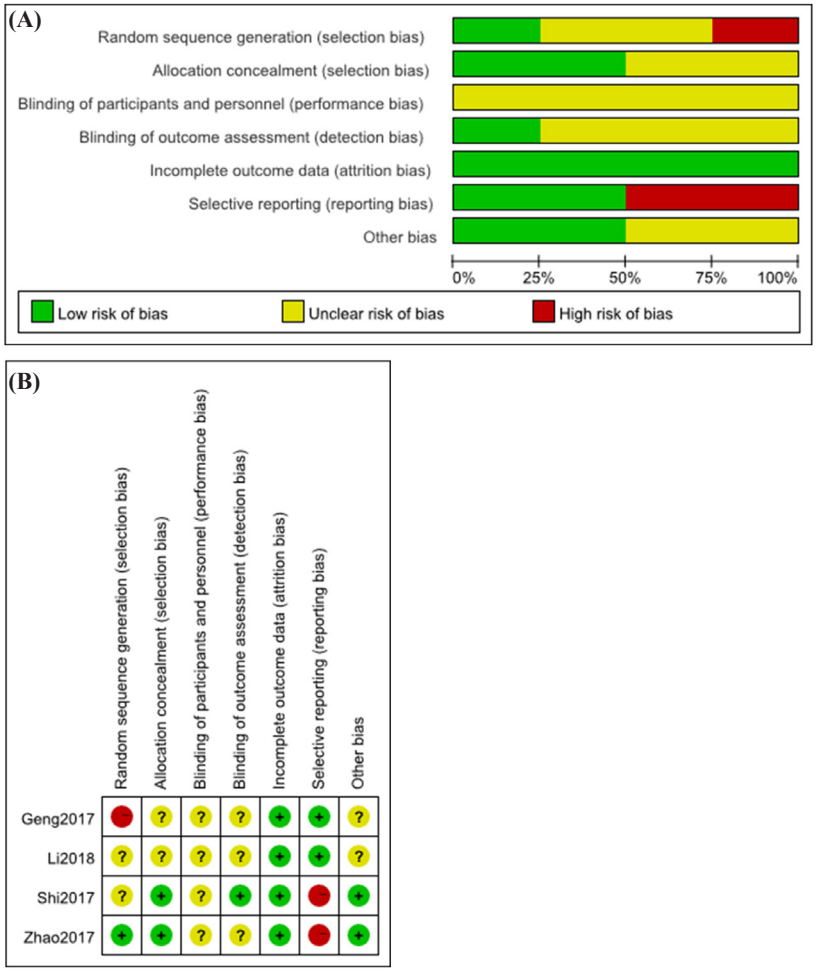

Figure 2. Risk of bias. (A) risk of bias graph; **(B)** risk of bias summary.

SP 6 stimulation for managing insomnia. It provides preliminary evidence of the effectiveness of single acupoint SP 6 for managing insomnia, although there were limitations to take into consideration.

Summary of main results

After combining the included four trials, the results showed that stimulation of SP 6 resulted in a better PSQI global score and sleep quality score. Meanwhile it may improve clinical effect and higher PSG scores than control interventions including moxa, multi-acupoints acupuncture, pseudo acupoint and sham. When it comes to the scores of sleep latency, sleep duration, sleep efficiency, sleep disturbance, and daytime function, there were no differences between stimulation of SP 6 and moxa on Shenmen acupoint.

In view of the results, stimulations on single acupoint SP 6 may improve sleep quality and lengthen deep sleep duration and REM duration of patients with primary insomnia and insomnia after surgery. The high heterogeneity may be because of the small number of trials and participants, different hospital settings, different ages of patients, different control interventions, lack of blinding, or difference characteristics of patients including education level and employment status. Hence more rigorous trials with high quality methodology and reporting should be performed in future clinical research.

The potential mechanism of acupoint stimulation for managing insomnia

Many acupoints could be used for managing insomnia, and the Evidence-Based Guideline in TCM for insomnia recommends SP6 as a main acupoint (*32*). Some potential mechanisms of stimulating acupoints for insomnia have been reported. Gamma-amino butyric acid (GABA), a main transmitter in the brain, has an inhibitory effect on neuroexcitability in the central nervous system (CNS). Lower GABA in the brain leads to many mental disorders including insomnia, and acupoint stimulation could increase GABA, which shows a similar effect with benzodiazepine (*33*). Acupoints stimulation also has an effect on controlling autonomic nervous system function such as regulating blood pressure and heart rate, which are disregulated in patients with insomnia (*34-36*).

Strength and limitations

Some limitations existed in this study. First, only

English and Chinese databases were searched because of language barriers. So it may have missed some high quality trials published in other languages (such as Japanese and Korean). Second, only two placebo-controlled trials (one used pseudo acupoint acupuncture and one was sham) were included in this review, which led to limited evidence from placebo-controlled trials. Third, the reporting and methodological quality of the included trials was low therefore the findings of this review should be interpreted with caution. Insomnia is reported as a long-term disorder mostly, while only four-week follow-ups were conducted in the included trials, so it is hard to conclude whether single acupoint stimulation on SP 6 had an effect on long-term insomnia. Finally, because of the small numbers of included trials, subgroup analysis and meta-analysis could not be conducted in this study, which may have a significant influence on the effectiveness of SP 6 for managing insomnia.

Implications for practice

This systematic review suggests that there is a lack of high-quality evidence to support single acupoint SP 6 stimulation for managing insomnia. Though there are limitations, SP 6 stimulation including acupuncture, warm-acupuncture, and acupoint paste may be recommended for insomnia, especially for primary insomnia. The recommended regimen durations may be from 20 min to 50 min lasting from 7 days to 25 days. The long-term effect of acupuncture is unknown and its potential adverse effects are not entirely clear.

Priorities for future research

Existing randomized controlled trials of SP 6 for managing insomnia are of small size and low methodological quality. Further high-quality trials of larger size are needed to assess the effectiveness of SP 6 for managing insomnia. Since insomnia is a highly heterogeneous condition with different etiologies and severity and acupoint stimulation is also a highly heterogeneous intervention, different stimulation at SP 6 is likely to have different effects on different subgroups of patients. Therefore, further clinical trials could focus on treating a particular subgroup using a particular stimulation at SP 6. Potential self-management approaches include stimulating SP 6 with acupressure, moxibustion, or other easy-to-do methods, which may be safe and easily accessible for insomniacs to perform at home at low cost. Further research is warranted to explore these potentials.

Conclusion

Some limited evidence shows that single acupoint stimulation of SP 6 could improve sleep quality, lengthen deep sleep and REM duration of patients with insomnia. However, the findings in this study should be interpreted with caution due to limitations in methodology. Better quality clinical trials are needed with precise methodology for single acupoint stimulation for managing all types of insomnia with pragmatic designs.

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REVIEW
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FDG-PET/CT images of COVID-19: a comprehensive review

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Abstract: Following a lot of reports of coronavirus disease 2019 (COVID-19) CT images, the feature of FDG-PET/CT imaging of COVID-19 was reported in several articles. Since FDG accumulates in activated inflammatory cells, FDG-PET/CT has huge potential for diagnosing and monitoring of inflammatory disease. However, FDG-PET/CT cannot be routinely used in an emergency setting and is not generally recommended as a first choice for diagnosis of infectious diseases. In this review, we demonstrate FDG-PET/CT imaging features of COVID-19, including our experience and current knowledge, and discuss the value of FDG-PET/CT in terms of estimating the pathologic mechanism.

***Keywords*:** COVID-19, FDG, PET/CT, diagnosis

Introduction

The coronavirus disease 2019 (COVID-19) outbreak that originated in Wuhan, China, spread across the world within a few months from the first report identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in January 2020 (*1-3*). Clinical manifestations and nucleic acid testing (RT-PCR) are essential in the diagnosis of COVID-19 (*4*). The common features of chest CT in patients with COVID-19 are multifocal patchy shadows and ground-glass opacities (*5,6*) (Figure 1). However, various diseases can mimic these features, including other viral pneumonias (*7*), and chest CT is therefore not currently recommended as an initial screening tool (*8*). Following numerous reports regarding the CT appearance of COVID-19, several groups have reported [^{18}F]-2-fluoro-2-deoxy-D-glucose (FDG) - positron emission tomography/computed tomography (PET/CT) imaging findings of COVID-19 (*9-16*). We experienced two measurements of FDG-PET/CT in a COVID-19 infected patient, one was 4 weeks from symptoms onset and the other was 4 weeks after the first FDG-PET/CT scan (Figure 2).

Here, we review the features of FDG-PET/CT, including our experience and the latest knowledge of COVID-19, and discuss the imaging findings to approach the pathological mechanism.

FDG-PET/CT

The glucose analog FDG is a molecular imaging probe used to evaluate tissue glucose utilization and glucose metabolism. A PET/CT test can provide metabolic and anatomic information of lesions simultaneously. FDG-PET/CT has utility in the staging, restaging, and assessment of therapeutic effects in malignancy, and is used in the management of patients with malignancy (*17*). Because FDG accumulates in activated inflammatory cells including neutrophils and macrophages, FDG-PET/CT has huge potential for diagnosing and monitoring inflammatory disease (*18*).

COVID-19 as an incidental finding

Patients with cancer and cardiovascular disease have a greater risk for worse clinical outcomes of COVID-19 infections (*19*). It is particularly noteworthy that the incidence of positive CT findings specific to COVID-19 was high among those who were asymptomatic but tested positive (*20*). Thus, departments treating patients with cancer and cardiovascular disease encountered high-risk patients with COVID-19, and imaging departments that possessed a CT scanner have a relatively high incidence of encountering highly suspicious findings of COVID-19 infection (*21*). In fact, some reports demonstrate incidental detection of COVID-19 infection in FDG-PET/CT examination in patients with malignancy (*11,21,22*). With regard to FDG-PET/CT, which is used in the management of patients with cancer, abnormal findings on chest CT and abnormal FDG uptake related to COVID-19 should be surveyed carefully, and we should be alerted immediately if COVID-19 infection is suspected.

As an advanced preparation, the nuclear medicine

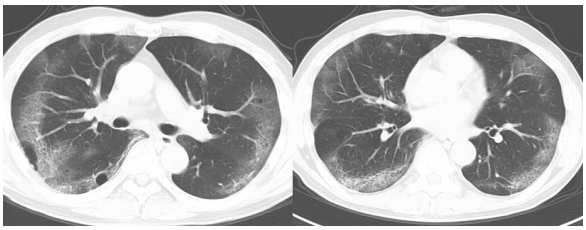

Figure 1. Chest CT images of COVID-19. Chest CT images at 14 days after symptom onset showed peripheral grand glass opacity with crazy-paving appearance in both lungs, which were typical findings of COVID-19 pneumonia.

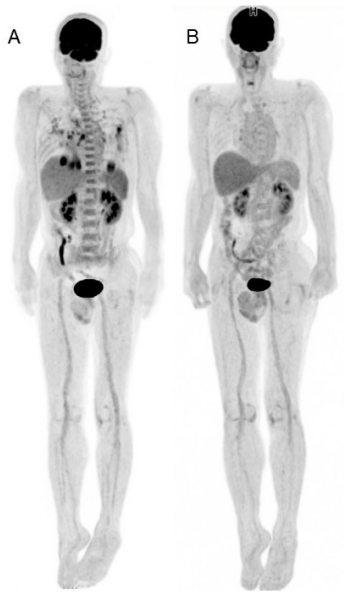

Figure 2. Whole-body FDG-PET images of COVID-19. (A): Whole body FDG-PET image of the patient with COVID-19 pneumonia (4 weeks after symptom onset and 3 weeks after negative RT-PCRs). **(B):** Whole-body FDG-PET image of the patient with COVID-19 pneumonia (4 weeks from previous FDG-PET scan). Intense FDG uptake was confirmed in lung lesion and mediastinal lymph node in the first image. In addition, increased FDG was seen in bone marrow and spleen. FDG uptake in lung lesion and mediastinal lymph node disappeared, and uptake in bone marrow and spleen were decreased as physiological uptake level.

department should have established effective procedures for patients and staff flow when facing known, suspected, and incidentally detected COVID-19 patients, and should control transmission of the virus while continuing to provide essential and critical services (*23*).

FDG-PET/CT requires a wait of at least 60 min after injection and approximately 20 min (depending on the machine) for scanning one patient. Therefore, patients with COVID-19 undergoing FDG-PET/CT would stay longer in the PET/CT department than in the CT scan room. The long-term care of COVID-19 patients in a small and closed space with limited equipment will be a burden for any staff in a PET/CT department. For this reason, FDG-PET/CT is not used routinely in an emergency setting and would not be the first choice for diagnosis of infectious diseases.

FDG-PET/CT imaging findings in COVID-19

Pneumonia

The most remarkable features of FDG-PET/CT in patients with COVID-19 are increased FDG uptake in lung lesions that form segmental ground-glass densities and plaques (*10,16,23*), which are typical CT findings in the early-stage of the disease. In non-small cell lung cancer (NSCLC), FDG uptake in the lesion correlates with tumor cell density and cell proliferation, thus early stage NSCLC featuring ground-glass opacities generally shows low FDG uptake (*24*). Therefore, FDG uptake of lung lesions in COVID-19 has an atypical appearance in terms of density-based considerations. Similar features have been confirmed in active interstitial pneumonia, in which FDG uptake reflects activity of the lesion (*25*).

SARS-CoV-2 infects cells expressing the surface receptors angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2). The active replication and release of the virus lead the host cell to undergo pyroptosis and release damage-associated molecular patterns. These patterns are recognized by neighboring epithelial cells, endothelial cells and alveolar macrophages, and trigger the generation of pro-inflammatory cytokines and chemokines (*26*). Thus, FDG uptake in segmental ground-glass density lesions suggests a high level of inflammatory related processes occurred in the lesion and looks like an early stage of COVID-19 in CT.

As a clinical progression of COVID-19, CT images reveal inflammatory exudation, consolidation, and increased density, accompanied by thickening of pulmonary vascular shadowing, bronchus sign, paving-stone sign, interlobular septal thickening, and pleural effusion (*12*). In most reports, FDG uptake was confirmed in progressed lung lesions in patients with common COVID-19 manifestation (*10,11,13-16*). It is well known that in pneumonia, intense FDG uptake appears in the active stage and during progression. Das *et al.* observed significant FDG uptake in progressed lesions such as lung nodules and cavities in patients with Middle East respiratory syndrome coronavirus (MERS-CoV) infection (*27*).

At the time of our first FDG-PET/CT scan, the respiratory symptoms of the patient were improved, and chest CT showed a reduction in the size of the segmental ground-glass opacities and plaques, decreasing lesion density, formulating liner opacity and trabecular shadowing were the same as the previous report (*28*). It is noteworthy that there was still intense FDG uptake in these CT features indicating the recovery stage of COVID-19 (Figure 3). In general, metabolic changes precede morphological changes; therefore, functional imaging using PET is a useful early predictor of the therapeutic response in inflammation and cancer lesions. Intense FDG uptake in lung lesions indicates

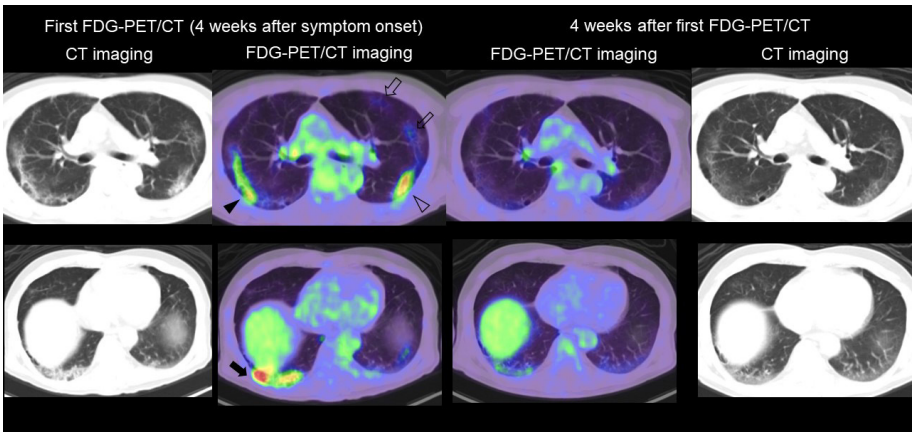

Figure 3. FDG-PET/CT imaging of lung lesion in COVID-19. Left side: CT and fused FDG-PET/CT image of the chest in the first examination (4 weeks after symptom onset). Right side: CT and fused FDG-PET/CT image of the chest (4 weeks after the first FDG-PET/CT examination). Intense FDG uptake was seen in liner opacity (black arrowhead), reticular opacity with consolidation (black arrow), and grand glass opacity with consolidation (open arrowhead) in the lung. Moderate FDG uptake was confirmed in grand glass opacity (open arrows) at the left upper lobe. All the FDG uptake in the first examination was significantly decreased in the second PET/CT scan.

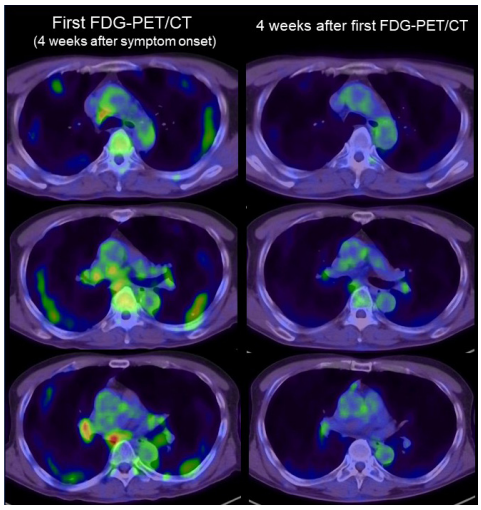

Figure 4. FDG-PET/CT imaging of lymph nodes in COVID-19. Left side: FDG-PET/CT image of the chest in the first examination (4 weeks after symptom onset). Right side: FDG-PET/CT image of the chest (4 weeks after the first FDG-PET/CT examination). Intense FDG uptake was seen in mediastinal and hilar lymph nodes. All the FDG uptake in the first examination was significantly decreased in the second FDG-PET/CT scan.

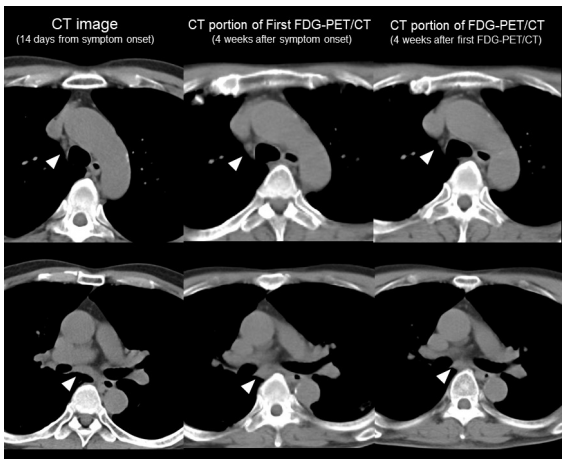

Figure 5. Change of CT feature of lymph node over time. Left side: CT image (14 days from symptom onset), Middle: CT portion of FDG-PET/CT (4 weeks after symptom onset), Right side: CT portion of second FDG-PET/CT (4 weeks after first FDG-PET/CT examination). CT image (14 days from symptom onset) showed no evidence of mediastinal lymph node swelling (arrowhead). Although the size of the lymph node is not significant, it was increased compared to 2 weeks from CT imaging and decreased within 4 weeks after the first FDG-PET/CT examination.

a high level of inflammatory change persists even in the recovery stage. However, it is still unclear whether the inflammatory change is caused by the remaining COVID-19 itself, the immunotherapeutic response, or angiovascular damage. In our experience, FDG uptake in lung lesions was decreased at 4 weeks after the first FDG-PET/CT scan (Figure 3). Therefore, we should note that FDG uptake in the recovery stage will not always forecast disease progression of COVID-19. Considering this finding, it is of interest whether FDG uptake can predict potential damage of lung tissue.

Lymph nodes

FDG uptake in lymph nodes is frequently seen in patients with COVID-19 (Figure 4). The FDG uptake in lymph nodes is thought to reflect immunoreactions activated by inflammatory cells such as neutrophils, monocytes, and effector T cells by the release of local chemokines. In the immune response to viral infections, the number of monocytes in lymphoid tissue increases, thus leading to increased FDG uptake (*12,29*).

A previous study reported that in COVID-19, lymph node enlargement is a rare finding on CT, which present in < 1% of patients (*30*). The size and the shape of these lymph nodes showing intense FDG uptake was not clear in some reports, but it is generally small, nonspecific, and regular in shape as we identified in our case (Figure 5). In our experience, FDG uptake was confirmed in mediastinal lymph nodes without significant

enlargement, and the uptake decreased during 4 weeks of observation (Figure 4). The CT image showed little change in size of a lymph node during the clinical course (Figure 5), but CT may be less sensitive to host reactions compared with FDG-PET/CT, and therefore the actual percentage of lymph node involvement may be higher than seen on CT.

In contrast, lymph node swelling has been manifested in pneumonia caused by parainfluenza virus and adenovirus (*30*). FDG uptake in small axillary lymph nodes is a common feature just after influenza vaccination (*31*). In COVID-19, however, several studies have reported negative FDG uptake in these lymph nodes, which may occur in the minimally invasive and early stages of the disease (*32*). Therefore, the immune response is weak or almost absent in the early stage and becomes more active over time. Moreover, reduction of FDG uptake in lymph nodes may indicate normalization of hyperactive immune response in the body, but further investigation is necessary to confirm this hypothesis.

Possible identification of lesions related to COVID-19

Patients with COVID-19 show various symptoms that can cause damage to the gastrointestinal tract, kidneys, heart, bone marrow, and other organs (*33*). Small vessel vasculitis causing skin disease (*34*) and symptoms similar to those of Kawasaki disease (*35*) have been reported as related to COVID-19. However, FDG-PET/CT is limited in its ability to diagnose small or middle vessel aortitis and medium-to-large vessel aortitis. In the case that vasculitis causes organ damage, the abnormal FDG-PET/CT findings on organs may indirectly indicate the existence of small-sized or middle-sized vessel aortitis (*36*).

Damage to endothelial tissue is considered to be the underlying mechanism of cardiovascular complications in COVID-19 (*37*). No report has described FDG uptake by the vascular wall that would suggest endothelial tissue damage. FDG can visualize metabolically active atherosclerosis because FDG is taken up by macrophages within atherosclerotic plaques (*38,39*). However, considering that complications of COVID-19 tend to occur in the elderly, it is questionable whether FDG can distinguish the uptake of FDG caused by atherosclerosis. Further investigation of the relationship between endothelial tissue damage and FDG uptake in the arterial wall in COVID-19 is required.

Because active thrombosis can be depicted as intense FDG uptake, a survey of FDG uptake when thrombosis is suspected may be of additional value in patients with COVID-19 (*40*).

Increased FDG uptake in bone marrow may be an additional imaging feature in COVID-19 as it was confirmed in another report (*9,13,15,16*). Chefer *et al.* reported high uptake by bone marrow over a long period of time in a MERS-CoV animal model (*41*). SARS-CoV-2 invades host cells *via* two receptors: angiotensin-converting enzyme 2 (ACE2) and CD147 (*42*). CD147 is expressed by mesenchymal stem cells of human cord blood and bone marrow origin (*43*), and CD147 expression is induced by high glucose concentration in monocytes (*44*). Based on this mechanism, FDG uptake by bone marrow may be an additional feature of COVID-19. In a COVID-19 patient, more neutrophils and scattered plasma cell infiltration are frequently found in the spleen. The author suggested that pathological changes of the spleen might be related to the direct attack of virus and the attack of immune cells (*45*). Similar to our case, slight to moderate FDG uptake in the spleen is confirmed in some reports (*10,13*), however the significance of this feature is still unknown. In another report, FDG-PET/CT imaging revealed hypoactivity of the orbitofrontal cortex in COVID-19 patients with anosmia (*46*).

Conclusions

We review the FDG-PET/CT imaging features of COVID-19, including our experience and current knowledge. FDG-PET/CT may have potential to increase our understanding of the mechanism of COVID-19. Further investigation is required to confirm the substantial value of FDG-PET/CT in patients with COVID-19.

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Lymphedema secondary to melanoma treatments: diagnosis, evaluation, and treatments

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Abstract: Approximately 300,000 new cases of melanoma are annually diagnosed in the world. Advanced stage melanomas require sentinel lymph node biopsy (SLNB), sometimes lymph node dissections (LND). The development rate of lower extremity lymphedema ranges from 7.6% to 35.1% after inguinal SLNB, and from 48.8% to 82.5% after inguinal LND. Development rate of upper extremity lymphedema ranges from 4.4% to 14.6% after axillary LND. Lymphedema management has constantly improved but effective evaluation and surgical management such as supermicrosurgical lymphaticovenular anastomosis (LVA) are becoming common as minimally invasive lymphatic surgery. Diagnosis and new classification using indocyanine green lymphography allowing pre-clinical secondary lymphedema stage management are improving effectiveness of supermicrosurgical LVA and vascularized lymph node transfer. Lymphatic transfer with lymph-interpositional-flap can restore lymph flow after large oncologic excision even without performing lymphatic anastomosis. Since lymphatic reconstructive surgery may affect local to systemic dissemination of remnant tumor cells, careful consideration is required to evaluate indication of surgical treatments.

***Keywords*:** lymphedema, melanoma, anastomosis, lymph node, supermicrosurgery

Introduction

Melanoma is an aggressive cutaneous cancer affecting 287,723 new patients and responsible for 60,712 deaths in 2018 in the world (*1*). Existence of metastatic regional lymph nodes is one of the most impairing factors on staging and survival prognosis (*2,3*). Sentinel lymph node biopsy (SLNB) is the standard procedure to determine the lymph node metastatic status and, according to the American and European recommendations, complete lymph node dissection (CLND) should be done if the sentinel lymph node is positive (*4,5*).

Despite these recommendations, this procedure is debated due to the morbidity of CLND and the limited oncologic benefit for some patients, but these conclusions are limited by inclusions bias (*6-10*). The SLNB and CLND morbidities are highly attributable to secondary lymphedema and its consequences such as chronic limb swelling responsible for discomfort and functional impairment, recurrent bacterial and fungal infection, ulcerations, psychosocial and cosmetic impairments (*11*). Also, several modifications of surgical technique have been suggested to reduce risk of complications, including preservation of the saphenous vein (*12,13*).

Despite these surgical improvements, lymph node dissections for melanoma treatment still lead to rates between 15.7% and 64.3% of secondary lymphedema (*14-18*). The purpose of this article is to focus on characteristics of secondary lymphedema after melanoma treatment and to report state-of-the-art secondary lymphedema treatments.

Extremity Lymphedema (EL) in melanoma

Lower extremity lymphedema (LEL)

European and American guidelines recommend regional lymph nodes dissection in the treatment of melanoma with positive sentinel lymph node (*4,5*). In the lower limb, the recommended lymphadenectomy is the femoro-inguinal lymph node dissection. SLNB is the standard procedure to determine lymph node metastatic status.

Lower extremity lymphedema (LEL) is the one of the most frequent complication of both SLNB and inguinal lymph node dissection (ILND) in melanoma. Reported rates of LEL secondary to inguinal SLNB were from 7.6% to 35.1% and from 48.8% to 82.5% after ILND (*16,19*). These rates are higher than the LEL rates reported after surgical treatment of advanced

pelvic cancers. According to studies, the LEL rate range after pelvic cancer is between 36.9% and 61% (*20-24*).

LEL in melanoma has a different physiopathology of the LEL secondary to lymphadenectomy for pelvic cancer, which is the cause of a higher rate of LEL. Indeed, American and European guidelines recommend pelvic lymphadenectomy and para-aortic lymphadenectomy in advanced gynecological and prostatic cancer (*25-31*). However, these lymphadenectomies preserve the superficial limb lymph nodes that are removed during the ILND for melanoma. Pelvic and para-aortic lymph node dissection are indirectly responsible for the obstruction of the lower limb superficial lymph flow whereas ILND for melanoma are directly responsible for it. The difference of LEL rates between melanoma and pelvic cancer seems to be due to the difference of lymph node dissection. The wide local excision seems to not be implicated in LEL, no study reported LEL after melanoma excision (*14-16,32*). However, no study compared lymph circulation patterns before and after local wide excision.

Upper extremity lymphedema (UEL)

Axillary lymph node dissection (ALND) is recommended for treatment for both advanced breast cancer and melanoma. The upper extremity lymphedema (UEL) rates after ALND for melanoma were from 4.4% to 14.6% in the reported studies and from 4.1% to 21.4% after ALND for breast cancer (*16-19,33-37*). These two rates are similar probably because the ALND is the same for the both cancers. ALND directly affects the upper limb superficial lymph flows. Also, no study compared lymphatic pattern before and after excision but the fact that these rates are similar is possibly due to the non-implication of wide local excision melanoma in UEL.

Diagnosis

Clinical manifestation of lymphedema

A heaviness sensation of the limb is the first manifestation of extremity lymphedema (EL). Extremity discomfort, tension, pain or tingling sensations can also be felt mostly during the evening. Edema can affect one, two or three limb parts depending on the EL stage. Other causes of edema such as heart failure, hepatic failure, nephrotic syndrome, cancer and venous insufficiency have to be excluded. In severe cases, EL can be associated with acute skin infections such as cellulitis and chronic inflammation causing skin thickening, interstitial tissue fibrosis, hyperkeratosis, and/or chronic ulcerations.

The International Society of Lymphology classification is based on physical condition of the extremities (*38*). Stage 0 refers to a latent or subclinical lymphedema without swelling. Stage I represents an early accumulation of fluid relatively high in protein content which subsides with limb elevation. Stage II signifies that limb elevation alone rarely reduces the tissue swelling and pitting is manifested except in late stage II when fibrosis is developed. Stage III encompasses lymphostatic elephantiasis where pitting can be absent and trophic skin changes are seen.

Imaging

Complementary imaging examinations in both primary and secondary lymphedema are fundamental. They help to confirm the diagnosis by showing involvement of a pathologic lymphatic system in edema, they allow to stage lymphedema and to schedule surgical procedures.

Indocyanine green (ICG) lymphography

ICG lymphography is used to study the superficial lymphatic system. Using a near infrared fluorescence camera, subcutaneously or intradermally injected ICG shows enhancement of fluorescent image of superficial lymph circulation up to 2 cm from the skin surface in real time. More related to the pathogenic mechanism of lymphedema, ICG lymphography severity staging has been developed based on the anatomy and the functional superficial lymphatic vessels as seen in ICG lymphography (Table 1) (*39*). Four patterns of ICG lymphography findings are correlated with clinical stage. Linear pattern (Figure 1A) is related to normally functional superficial lymphatic collectors. When lymph flows are obstructed, the lymphatic collectors become dilated, leading to retrograde lymph flows called dermal backflow.

The first and less severe dermal backflow pattern is the splash pattern (Figure 1B) that is correlated with lymphatic reflux into the more superficial collecting and precollecting lymphatic vessels, showing tortuous lines on ICG lymphography. The second dermal backflow pattern is the stardust pattern (Figure 1C) correlated

Table 1. Pathophysiolgical severity stage based on ICG lymphography findings

ICG stage	Lymphography findings	Clinical conditions
stage 0	Linear pattern only (no DB pattern)	No lymphedema
stage I	Splash pattern (+ Linear pattern)	Subclinical lymphedema
stage II	SD pattern in 1 region (+ Linear pattern)	Early lymphedema
stage III	SD pattern in 2 region (+ Linear pattern)	Progressed lymphedema
stage IV	SD pattern in 3 region (+ Linear pattern)	
stage V	SD pattern only (no Linear pattern)	

Upper/lower extremity can be divided into 3 regions; the upper-arm/thigh, the forearm/lower-leg, and the hand/foot. ICG, indocyanine green; DB, dermal backflow; SD, Stardust and/or Diffuse.

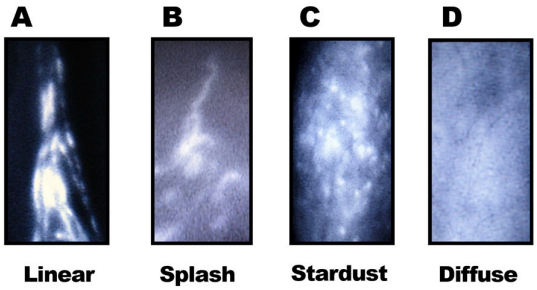

Figure 1. Characteristic ICG lymphography patterns. (A) Linear pattern; **(B)** Splash pattern; **(C)** Stardust pattern; **(D)** Diffuse pattern. Lymphographic pattern changes from Linear to Splash, Stardust, and finally to Diffuse pattern with progression of lymphedema.

with lymphatic reflux into the precollecting lympahtic vessels flowing vertically to the dermal capillary lympahtics, showing spots on ICG lymphography. The most severe dermal backflow is the diffuse pattern (Figure 1D) related to lymph flows in the dilated dermal lymphatic capillaries, showing a diffusely enhanced area on ICG lymphography (*40-44*).

Lymphoscintigraphy

Lymphoscintigraphy is described as the principal examination to perform in lymphedema. Lymphoscintigraphy can be used to study the superficial and deep lymphatic system whereas ICG lymphography can only study the superficial lymphatic system. Lymphoscintigraphy informs about qualitative and quantitative functional parameters of the lymphatic system. Normal lymphoscintigraphy shows normal superficial and deep lymphatic vessels and lymph nodes as part of these pathways. Abnormal lymphoscintigraphy can show, lack of lymphatic vessels (superficial or deep), lack of lymphatic nodes, post-obstruction reflux into the lymphatic collateral network or dermal backflow (diffuse tracer repartition) in one part of the limb or through the whole limb. Axillary or groin lymphorrhea can be observed after lymph node dissection, slow (persistence of tracer activity) or incomplete lymphatic drainage. Some authors reported lymphoscintigraphy based classifications to select the most appropriate surgical treatment (*45*).

Single photon emission computed tomography-computerized tomography (SPECT-CT) lymphography and Magnetic Resonance Lymphography (MRL)

SPECT-CT lymphography and MRL can also give information about superficial and deep lymphatic systems. One of the most interesting advantages is that they can give volumetric details and localize superficial and deep lymphatic channels seen as linear or tortuous vessels and lymph nodes (*46*). They can also give precise localization and function of the collateral network. Contrast diffusion can, as in ICG lymphography, reveal dermal backflow. However, injections are required and, recurrent irradiation in post-cancer patients should be limited. Moreover, regarding lymphatic disease progression or post-surgical evolution and their availability, some centers cannot afford to repeat these examinations.

Lymphedema management

Non-surgical Management

Medical management

No medical treatment is indicated in routine management of lymphedema however, some studies reported an effect of medical treatment on EL. Results of studies focusing on coumarin, diosmin and arbutin are contrasted and, due to hepatotoxicity, there is no recommendation for routine use (*47,48*). Diuretics are considered a contraindication. Antibiotics are recommended to prevent recurrence of limb cellulitis but are not effective for lymphedema. The overuse of antibiotics increases the risk of emergence of multi-drug resistant bacterial infection.

Decongestive therapy

Physiotherapeutic management of EL has been reported to be effective. Studies proved that pneumatic compression are effective on EL (*49-52*). Treatment of lymphedema with complete decongestive physiotherapy (CDT), which combines manual lymphatic drainage, lymphedema rehabilitation exercises, compression therapy, and skin care, can achieve a 45-70% reduction in EL volume (*48-50*). Phase 1 of CDT consists of skin care and manual lymphatic drainage. Phase 2 consists of compression, manual drainage and exercises to conserve the benefit obtained in phase 1.

Magnetotherapy and electrotherapy have also shown good results (*53*). Intermittent pneumatic compression has shown good results but only a few studies have been published. It is understood that compression therapy used in CDT is effective in EL treatment but has to be adapted so as not to reduce the quality of life (*54-56*). Thermal therapy, aquatherapy, low-level laser therapy and ultrasounds therapy have also been suggested.

All of this non-surgical management is anti-symptomatic treatment and not curative, because it cannot restore lymph flow. Therefore, life-long treatment is required.

Surgical management

EL surgical treatment includes several procedures which can be separated into two groups: physiological and ablative surgeries. Physiological surgeries aim to restore lymph drainage to the lymphatic system, venous system or new lymphatic pathways after lymphangiogenesis

whereas ablative surgeries remove affected tissues (*36,57-59*). Physiological surgeries are so classified:

• Lymphatic bypasses which aim to divert congested lymph to intact lymphatic or venous circulation. They can be classified into lymphatico-lympahtic bypass, lymphatico-venous implantation, lymph node to vein shunt and lymphaticovenular anastomosis.

• Lymphatic transfers from a healthy lymphatic donor site. They include vascularized lymph node transfer with or without efferent lymphatic vessel anastomosis, and lymph-interpositional-flap transfer (LIFT).

Lymphatic bypass

Lymphatico-lymphatic bypass: Lymphatic to lymphatic bypass, using a lymphatic graft seems more physiologic. It has been reported on a 329 patient series that more than 60% of the patients with UEL showed a reduction in volume difference to the healthy side of more than 50% after a mean follow-up period of more than 2 years (*60*). However, this technique is invasive for the donor site with a risk of lymphedema on the donor site.

Lymph node to vein anastomosis and lymph node implantation: The implantation uses microsurgical techniques to insert lymphatic vessels into a vein. Some authors reported good results but the thrombosis risk is higher than in supermicrosurgical lymphaticovenular anastomosis (LVA) because the lymphatic vessel adventitia is in contact with the venous lumen (*61-63*). Serious complications such as deep venous thrombosis and pulmonary embolism were reported. Because of high risk of thrombosis and possibility of serious sequelae, this procedure has been abandoned by most lymphatic surgeons.

Supermicrosurgical lymphaticovenular anastomosis (LVA): Unlike the above mentioned classical lymphovenous shunt operations, supermicrosurgical LVA creates a real anastomosis of lymphatic vessel to recipient venule or small vein in an intima-to-intima coaptation manner. Since lymphatic vessels can be smaller than 0.5 mm, supermicrosurgical techniques which allow anastomosis of vessels with an external diameter of 0.5 mm or smaller, is necessary to perform LVA surgery. LVA are an anastomosis between a superficial lymphatic vessel (mostly under the superficial fascia) and a superficial vein. Supermicrosurgical anastomosis allows intima-to-intima coaptation even when vessel diameters are smaller than 0.5 mm.

LVA is performed in an end-to-end, side-to-end, side-to-side, or end-to-side fashion. Various anastomotic configurations can be combined to maximize lymph flow drainage. Lambda-shaped LVA allows bidirectional bypass using a lymphatic vessel and a vein with end-to-end and end-to-side anastomosis (*64-72*). LVA is the least invasive surgery to treat lymphedema. It can be performed under local anesthesia through an approximately 2 cm incision allowing for day surgery.

Lymphatic transfer

Vascularized lymph node transfer (VLNT): VLNT is a reconstructive lymphatic surgery mainly for advanced cases where lumen of lymphatic vessels are obstructed because of lymphosclerosis or patients where lymphatic vessels are not found. VLNT requires less technically demanding procedures, since supermicrosurgy is not basically needed (*73,74*). Supermicrosurgery is required, when the efferent lymphatic vessel of a transferred lymph node is anastomosed (*58*).

Two different mechanisms are suggested to explain VLNT effects. The first one is that VLNT would act like a bridge over the obstruction zone because the VLNT flap contains many functional lymphatic vessels and nodes. Therefore, the VLNT flap has to be large enough to reach both beyond the obstruction, and to reconnect lymphatics on both sides *via* lymphangiogenesis (*74*). The other mechanism is that the VLNT flap would act like a lymphatic pump to the blood circulation (*75*). Several donor sites have been identified; inguinal, lateral thoracic, supraclavicular submental and omentum. On a literature review of about 271 VLNT cases (24 studies), Scaglioni *et al.* reported that submental VLNT were the most effective with 100% of patients showing improvement, the supraclavicular was the second highest rate of benefit (88.2%), followed by the inguinal VLNT (70.4%, n =138), 60% of omental VLNT demonstrated benefit, and only 5% of lateral thoracic VLNT reported an improvement. The highest complication rate on donor site was on lateral thoracic (15.8%), then in inguinal (10.9%), supraclavicular (1.2%) and submental (0%) and omentum (0%). Donor site lymphedema was more frequent in lateral thoracic (13.2%) and inguinal (1.6%). No donor site lymphedema was reported on supraclavicular, submental or omentum.

Lymph-interpositional-flap transfer (LIFT): Traumatic lesions and oncologic excisions can interrupt lymphatic flow and lead to lymphedema. A retrospective study suggested that tissue replantation or reconstruction could restore lymph flow without lymph node transfer or lymphatic vessel anastomosis (*59*). This study showed that spontaneous lymph flow restoration depended on compatible lymph axiality without raw surface in lymph axiality. When lymphatic vessel stumps in a recipient site and transferred tissue were approximated to each other, the lymphatic vessels could be reconnected spontaneously without supermicrosurgical lymphatic anastomosis. Based on the concept of lymph axiality, a new lymphatic reconstruction, LIFT, has been developed, allowing lymph flow reconstruction without supermicrosurgical technique or lymph node sacrifice. Since LIFT does not sacrifice lymph nodes at the donor site, donor site lymphedema risk is significantly reduced unlike VLNT.

For LIFT operation, ICG lymphography is necessary to precisely localize lymphatic vessels both in a donor flap and a recipient site. Linear patterns from flaps were

aligned as best possible to the donor site linear patterns under ICG lymphography surgical navigation. LIFT can be applied for primary prevention of lymphedema in oncological ablative surgery and for treatment of established secondary lymphedema.

Debulking surgeries: resection and liposuction

Chronic lymphedema is responsible for damaging soft tissues and leads to a dermato-lipofibrosclerosis. Patients with severe stage lymphedema can be affected by recurrent fungal and bacteriologic infections, as well as elephantiasis and have a deformed extremity limiting compression effectiveness. Once fat deposition and fibrotic histopathological changes occur, reconstructive surgery cannot improve the changes, and some debulking procedures may be required to improve the established histopathologic changes. Unlike lymphatic reconstructive surgery, debulking surgeries aim to decrease lymphedematous volume directly by removing the lymphedematous tissue, allowing an immediate affect of volume reduction. However, debulking procedures destroy the remaining lymphatic structures, and worsen lymph circulation. Therefore, even stronger compression treatment is required after some debulking surgeries.

Charles' procedure was a surgical excision management described in 1912. The treated limb part (thigh or thigh+leg) is circumferentially denuded down to the deep fascia. The deep fascia thickness is also reduced to a normal size. The excised tissue is used as a donor site for split thickness skin graft. Feins described a Hofman's procedure. Skin incision is done from up to down in the affected limb. Two skin flaps are harvested. Lymphadematous tissues from subcutaneous fat to deep fascia are excised. After hemostasis, skin flaps are replaced on muscles. Depending on volume excess, this procedure can be repeated (*76*). Some authors describe improvement of clinical conditions and quality of life after excisional surgery or liposuction on severe lymphedematous patients (*77,78*). This type of surgery should be considered only after failure of all physiologic treatments and only when patient's compliance for maximum compression therapy is confirmed, because lymphedematous tissues shall re-increase as lymph circulation is even further deteriorated after debulking procedures; debulking procedures can also be destructive to remaining lymphatic functions.

Conclusion

Secondary limb lymphedema after SLNB or CLND for melanoma affects a high percentage of patients and lymphatic follow-up should systematically be considered. Lymphatic surgeries after melanoma may present a possible risk to accelerate dissemination of a local melanoma recurrence, which should be well evaluated before performing lymphatic surgeries.

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Identifying medical professionals at risk for in-hospital COVID-19 infection: a snapshot during a "tsunami" highlighting unexpected risks

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Abstract: The aim of this study was to profile healthcare professionals (HCPs) who infected with COVID-19 in hospital while working in a COVID-19 hub hospital during the pandemic. A questionnaire was sent to all HCPs from whom nasopharyngeal swabs (NPS) were collected. The type of work, work environment, individual characteristics, and modality of infection were analyzed. Working areas were categorized into COVID-free areas (wards and ICUs for patients without COVID-19, medical offices, and hospitality counters) and COVID+ areas (dedicated wards and the ICU for patients with COVID-19). From March 1 to 20, 2020, 302 HCPs were tested: 251 (83.1%) responded to the questionnaire, but 9 were excluded since infection occurred outside the hospital. The remaining 242 subjects included 53 (21.9%) with positive NPS and 189 (78.1%) with negative NPS, significant differences in NPS results were evident depending on the subject's role (p = 0.028). Pairwise post hoc analysis revealed that surgeons had a significantly increased rate of positive NPS (p = 0.001). Of the 189 subjects with negative NPS, 175 (92.6%) worked in COVID-free areas, and 14 (7.4%) in COVID+ areas. Of the 53 subjects with positive NPS, 44 (83.1%) worked in COVID-free areas and 9 (16.9%) worked in COVID+ areas. Medical offices featuring an open space with adjacent desks were identified as areas of higher risk. An apparent cause of infection could not be identified in 21 (39.6%) subjects with positive NPS. Among a total of 251 subjects, 80 (41.5%) of the 193 subjects with negative NPS and 16 (27.6%) of the 58 subjects with positive NPS had been vaccinated against the common flu. In conclusion, the vast majority of subjects with positive NPS came from COVID-free areas. The source of infection could not be identified in a significant portion of subjects with positive NPS. Personnel need better protection, more testing with NPS needs to be performed, and workplace layouts need to be re-thought. Vaccination against the flu seems to provide some protection.

Keywords: COVID-19, SARS-CoV-2, healthcare personnel, healthcare system

Introduction

Since the COVID-19 outbreak caused by SARS-CoV-2, hospitals have managed a high number of critically ill patients, posing a challenge to healthcare systems worldwide (*1-5*). In Europe, Italy was hit first, the impact has rapidly spread, with Lombardy and Veneto being the two most affected regions. Lombardy suffered from a huge number of patients, overwhelming the healthcare system's capability to provide care, despite being one of the most efficient regions within the Italian National Health Service (NHS). A high incidence of COVID-19 has been reported among healthcare professionals (HCPs), over 10,000 HCPs have been infected and over 150 physicians have died (*6*).

Profiling HCPs in terms of specialty and working areas within the hospital would help to identify risks, readdress the need for protection, better highlight the need for testing, and foreshadow new working environments. With these aims in mind, the current study profiled HCPs who infected with COVID-19 in hospital.

Materials and Methods

Overall design

A survey was conducted at the Humanitas Research Hospital (HRH), which has 750 beds, 31 of which

are dedicated to the intensive care unit (ICU). Among 2,580 HCPs working in this hospital, 540 are physicians (including residents), 200 are surgeons (including residents), 75 are anesthesiologists (including residents), 1,140 are nurses and patient care technicians (PCTs), 70 are radiologic technicians (RTs), and 550 are administrative staff.

During the pandemic, HRH handled 260 patients with COVID-19 (47 in the ICU) and 220 without COVID-19. According to regional government guidelines, nasopharyngeal swabs (NPS) were initially collected only from subjects with symptoms suggestive of COVID-19 or after exposure to individuals confirmed to have COVID-19. After March 12, 2020, medical masks became available to all HCPs, given the increased number of individuals with COVID-19, NPS were collected only from symptomatic HCPs.

From March 1 to 20, 2020, 302 HCPs were tested and surveyed.

Statistical analysis

Data were reported as the number and percentage or the median and interquartile range (IQR) as appropriate. Comparisons were made using the chi-square or Mann-Whitney tests. Pairwise post hoc analysis was performed with Bonferroni correction to identify significant variables. Analysis was performed with the software R (version 3.6.1).

Results

Two hundred and fifty one (83.1%) subjects responded to the questionnaire, including 58 with positive NPS and 193 with negative NPS. Nine subjects were excluded from analysis since they reported that they contacted with a COVID-19 patient occurred outside the hospital. The 242 remaining subjects included 53 (21.9%) with positive NPS and 189 (78.1%) with negative NPS.

Of the 242 remaining subjects, females accounted for 56.6% of the subjects with positive NPS and 73.0% of the subjects with negative NPS. No significant differences between the two groups were evident in terms of age or comorbidities (Table 1). Flu-like symptoms were present in 86.8% of subjects with positive NPS and 52.4% of subjects with negative NPS ($p < 0.001$). Only 3 (5.7%) subjects with positive NPS required hospitalization. None of the subjects with negative NPS developed COVID-19.

The 78 physicians (32.2%) included 52 internists, 22 surgeons, and 4 anesthesiologists. Of the total subjects, 74 (30.6%) were nurses, 21 (8.7%) were PCTs, 5 (2.0%) were RTs, 25 were (10.3%) secretaries at hospitality counters, and 39 (16.1%) performed some other role. Significant differences in NPS results were evident depending on the role of HCPs in the hospital ($p = 0.028$). Of the 53 subjects with positive NPS, most (20 subjects, 37.7%) were physicians (11 (55.0%) of whom were surgeons), followed by nurses (28.3%), PCTs (11.3%), and subjects in another discipline as shown in Table 1.

Working areas were categorized in COVID-free areas (wards and ICUs for patients without COVID-19, medical offices, and hospitality counters) and COVID+ areas (dedicated wards and the ICU for patients with COVID-19). Of the 189 subjects with negative NPS, 175 (92.6%) worked in COVID-free areas and 14 (7.4%) worked in COVID+ areas. Of the 53 subjects with positive NPS, 44 (83.0%) worked in COVID-free areas and just 9 (17.0%) worked in COVID+ areas.

Table 2 shows NPS results cross-referenced with working areas and profession. Pairwise post hoc analysis revealed that surgeons had a significantly increased rate of positive NPS ($p = 0.001$). All 7 internists with positive NPS spent the majority of their time in COVID-free wards and the outpatient clinic. Six (54.5%) of the 11 surgeons with positive NPS did the same, while 5 (45.5%) spent more time or had contact with subjects with COVID-19 in medical offices that largely featured an open space with adjacent desks. The 2 anesthesiologists with positive NPS both worked in a COVID-free area. Nine (60.0%) of the 15 nurses with positive NPS worked in a COVID-free area while 6 (40.0%) worked in COVID+ areas. One PCT with positive NPS worked in a COVID+ area while the remaining 5 (83.3%) worked in COVID-free areas, as did all 6 cleaners and the 4 secretaries at hospitality counters with positive NPS. The one RT with positive NPS was also the only subject to be infected in the COVID+ ICU.

Among the 242 subjects analyzed, 195 declared a contact with a potential source of infection: 89 had contact with a colleague who infected with COVID-19, and 106 had contact with a patient with COVID-19. An apparent cause of infection could not be identified in 47 subjects. Figure 1 details the relations between positive NPS, and potential cause of infection. Among the 53 HCPs with positive NPS, 20 (37.7%) and 12 (22.6%) referred to a patient and a colleague as the cause of infection, respectively, while 21(39.6%) could not identify any apparent cause of infection.

Other factors besides work-related transmission were investigated. Of note, among a total of 251 subjects, 80 (41.5%) of the 193 subjects with negative NPS and 16 (27.6%) of the 58 subjects with positive NPS ($p = 0.151$) had been vaccinated against the common flu (Figure 2A). Three (5.2%) of the subjects with positive NPS were hospitalized, and none of them were vaccinated against the common flu (Figure 2B).

Discussion

Since February 18, 2020 when the infection occurred in Lombardy to April 11, the total number of cases in Italy exceeded 100,000 and there were approximately

Table 1. Characteristics of clinical data from the surveyed HCPs categorized by NPS results

Items	Negative NPS	Positive NPS	*p*
N	189	53	
Female (%)	138 (73.0)	30 (56.6)	0.034
Age (years) (median [IQR])	41.00 [32-48]	41.00 [33-46]	0.722
Symptoms before NPS (%)	99 (52.4)	46 (86.8)	< 0.001
Hospitalization (%)	0 (0.0)	3 (5.7)	0.010
Role of HCP (%)			0.028
Internist	45 (23.8)	7 (13.2)	
Surgeon	11 (5.8)	11 (20.8)	
Anesthesiologist	2 (1.1)	2 (3.8)	
Nurse	59 (31.2)	15 (28.3)	
PCT	15 (7.9)	6 (11.3)	
RT	4 (2.1)	1 (1.9)	
Administrative	21 (11.1)	4 (7.5)	
Other	32 (16.9)	7 (13.2)	
Hospital area (%)			
COVID+ area	14 (7.4)	9 (17.0)	
COVID-free area	175 (92.6)	44 (83.0)	
Smokers (%)	41 (21.7)	8 (15.1)	0.388
Vaccinated against season flu (%)	80 (42.3)	16 (30.2)	0.151
Absence of coronary disease (%)	189 (100.0)	53 (100.0)	NA
Peripheral vascular disease (%)	4 (2.1)	1 (1.9)	1.000
Ictus / TIA (%)	1 (0.5)	0 (0.0)	1.000
Chronic obstructive pulmonary disease (%)	5 (2.6)	0 (0.0)	0.516
Mild hepatitis (%)	1 (0.5)	0 (0.0)	1.000
Diabetes (%)			0.298
Absent or controlled by diet	185 (97.9)	51 (96.2)	
Diabetes without organ damage	2 (1.1)	2 (3.8)	
Diabetes with organ damage	2 (1.1)	0 (0.0)	
Chronic kidney disease (%)	2 (1.1)	0 (0.0)	1.000
Local cancer (%)	1 (0.5)	1 (1.9)	0.915
Leukemia (%)	2 (1.1)	0 (0.0)	1.000
Lymphoma (%)	3 (1.6)	0 (0.0)	0.825
AIDS (%)	2 (1.1)	0 (0.0)	1.000
CCI (median [IQR])	0.00 [0.00, 1.00]	0.00 [0.00, 0.00]	0.394

CCI, Charlson comorbidity index; HCP, healthcare professional; NPS, nasopharyngeal swab; PCT, patient care technicians; RT, radiologic technicians.

Table 2. NPS results by working area and profession

Areas/Discipline	Negative NPS		Positive NPS		*p*	pairwise *p*
	N	%	*N*	%		
COVID-free area						
Internist	45	25.7	7	25.7	0.006	
Surgeon	11	6.3	11	6.3		< 0.001
Anesthesiologist	2	1.1	2	1.1		
Nurse	49	28.0	9	28.0		
PCT	11	6.3	5	6.3		
RT	4	2.3	0	2.3		
Administrative	21	12.0	4	12.0		
Other	32	18.3	6	18.3		
COVID+ area						
Nurse	10	71.4	6	71.4	0.273	
PCT	4	28.6	1	28.6		
RT	0	0	1	0		
Other	0	0	1	0		

NPS, nasopharyngeal swab; PCT, patient care technician; RTs, radiologic technician.

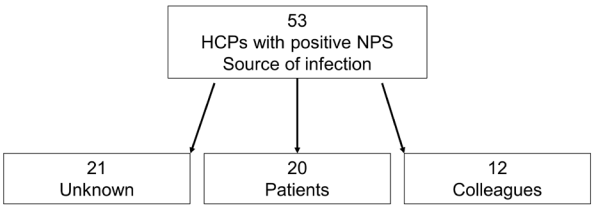

Figure 1. The source of infection for 53 HCPs with positive NPS. HCPs, healthcare professional; NPS, nasopharyngeal swabs.

20,000 total deaths (*7*). These conditions allowed no other option than to look at the problem immediately at hand. Thus, efforts have been focused on supporting COVID-19 wards and the ICU. As the survey results demonstrate, front-line HCPs in contact with patients with COVID-19 have been properly protected. However, the pandemic has been compounded by the global shortage of personal protective equipment (PPE). The WHO at that time was recommending the rational use of PPE in healthcare and home care settings (*8*). Given the scarcity of PPE in the supply chain, its acquisition was chaotically marked by panic buying and stockpiling, as a consequence, the demand for PPE even in hospitals could not be satisfied. This hampered

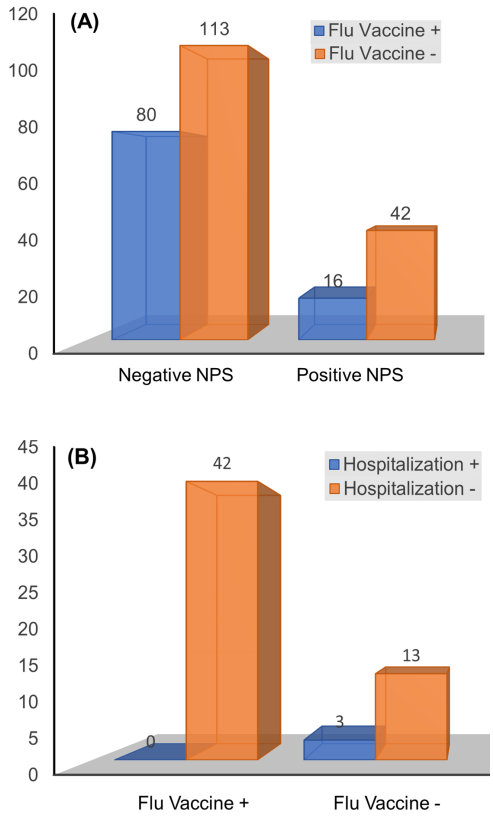

Figure 2. (A) number of patients who had NPS and were vaccinated or not against the common flu; (B) number of patients NPS positive, categorized based on common flu vaccination status who were hospitalized or not. NPS, nasopharyngeal swabs.

the equipping of HCPs with PPE as recommended (*9*).

At this hospital, the situation stabilized on March 12, 2020. Possibly, the lack of PPE is one reason why all but one of the HCPs with positive NPS was working in COVID-free areas. However, the layouts of working areas may have been part of the problem, too. Of note, surgeons represented 9% of the subjects tested but accounted for over 20% of subjects infected with COVID-19 and over 50% of physicians with COVID-19. That occurred during a time frame in which surgical activity decreased significantly to allow the hospital to focus on the outbreak. Therefore, surgeons dedicated more time to research and spent more time in offices featuring an open space with adjacent desks, which inevitably facilitated the transmission of the virus. Given the presumably prolonged duration of this pandemic and the increased incidence of zoonosis accentuated by globalization, the current findings should induce facilities, and especially hospitals, to reconsider the layouts of working areas.

Another issue is limiting testing to symptomatic individuals. The regional government guidelines limited the collection of NPS to symptomatic patients. The lack of tools for testing and concern about the limited value of NPS results may have played a role in prompting that restriction.

Nonetheless, the current survey revealed that 40% of subjects with positive NPS had not been warned about significant contact with asymptomatic carriers. Screening of HCPs, which presumably will benefit from the introduction of and improvements in blood tests (*10*), and other diagnostic tools (*11*), is certain to play a crucial role in the near future once hospitals reassess the need to preemptively isolate areas for patients with COVID-19 from areas for patients without COVID-19. This is particularly relevant because of the need for prompt and appropriate care for patients with other conditions, like cancer, whose treatment was unfortunately delayed during the pandemic (*12*).

A final point worth mentioning is the extent of flu vaccination among the HCPs surveyed. Survey results indicated that vaccination against the common flu seems to provide some protection against COVID-19. The current findings are speculative and require further investigation, but HCPs might be encouraged to be vaccinated against the flu.

The limitations of this study are readily acknowledged. However, this study can help to understand an otherwise unpredictable phenomenon. The pandemic was a kind of "tsunami" where decision-making processes were also influenced by external factors. Nonetheless, results revealed that the profile of HCPs and their working areas were major risks factors for infection. These data could support and enhance preemptive recommendations (*8*).

In conclusion, a healthcare system is obviously placed under significant strain because of a pandemic, and concerns about in-hospital COVID-19 infection could further undermine its ability to address the emergency. Looking toward the future and in light of experiences combatting this pandemic (*13*), the categories of COVID+ and COVID-free areas should be reassessed in the healthcare system. This study has provided data highlighting the need to pay more attention to HCPs working in COVID-free areas. Essential approaches to dealing with that risk are providing adequate PPE, providing working areas that allow social distancing, and implementing more efficient screening policies to identifying asymptomatic individuals.

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Impact of sex and histology on the therapeutic effects of fluoropyrimidines and oxaliplatin plus bevacizumab for patients with metastatic colorectal cancer in the SOFT trial

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Abstract: Mechanisms accounting for sex differences in the incidence of adverse events caused by fluoropyrimidine treatments, and histologic differences in efficacy are insufficiently understood. We determined differences between the sexes in terms of the safety of S-1 plus oxaliplatin (SOX)/bevacizumab-versus-l-leucovorin, 5-fluorouracil (5-FU) and oxaliplatin (FOLFOX)/bevacizumab, and the impact of histology on their therapeutic effects, in 512 unresectable metastatic colorectal cancer patients from the SOFT phase III study. Nausea (OR: 2.88, $P < 0.001$) and vomiting (OR: 3.04, $P = 0.005$) occurred more frequently in females than males treated with SOX/bevacizumab, while nausea (OR: 2.12, $P = 0.006$), vomiting (OR: 3.26, $P = 0.004$), leukopenia (OR: 2.61, $P < 0.001$), neutropenia (OR: 2.92, $P < 0.001$), and alopecia (OR: 4.13, $P < 0.001$) were higher in females on FOLFOX/bevacizumab. Mean relative dose intensities (RDIs) of S-1 during all cycles of SOX/bevacizumab were significantly lower in females (73.9%) than males (81.5%) ($P < 0.001$), while RDIs of continuous infusion of 5-FU in the FOLFOX/bevacizumab regimen were 75.0% in females and 80.5% in males ($P = 0.005$). No significant differences in efficacy with regard to overall survival (OS) and progression-free survival (PFS) were identified between the sexes for either SOX/bevacizumab or FOLFOX/bevacizumab treatment. Patients with poorly-differentiated adenocarcinoma had significantly worse OS (HR: 2.72, 95% CI: 1.67-4.44, $P < 0.0001$) and PFS (HR: 1.89, 95% CI: 1.18-3.02, $P = 0.0079$) than patients with well- or moderately-differentiated adenocarcinoma. Female patients experienced more frequent and severe adverse reactions to SOX/bevacizumab and FOLFOX/bevacizumab and a worse prognosis for poorly-differentiated adenocarcinoma were confirmed in this phase III study. This warrants further translational research to identify the responsible mechanisms.

***Keywords*:** gender, fluorouracil, S-1, poorly differentiated adenocarcinoma, bevacizumab

Introduction

Colorectal cancer is the second leading cause of cancer-related deaths worldwide (*1*). Fluoropyrimidines and their biochemical modulators have been key drugs used in strategies for treating patients with metastatic colorectal cancer for more than 6 decades. FOLFOX (leucovorin, 5-fluorouracil (5-FU), and oxaliplatin) or FOLFIRI (leucovorin, 5-FU, and irinotecan) plus bevacizumab have been widely used as first-line treatment options for metastatic colorectal cancer (*2,3*). The SOFT trial showed that oral S-1 and oxaliplatin

(SOX) plus bevacizumab was non-inferior to FOLFOX plus bevacizumab and the TRICOLORE (*4*) study showed that S-1 and irinotecan plus bevacizumab was non-inferior to FOLFOX or capecitabine/oxaliplatin (CapeOX) plus bevacizumab for progression-free survival (PFS), thereby establishing the therapeutic usefulness of this agent (*5-7*). S-1 is an oral anticancer drug that combines tegafur, a prodrug of 5-FU, with two modulators. The first of these, gimeracil, reversibly inhibits DPD, the primary metabolizing enzyme of 5-FU, and thus maintains higher 5-FU levels in the blood for a longer period of time. The second is oteracil potassium, which suppresses, and thereby decreases, the activity and toxicity of 5-FU for normal gastrointestinal tissue (*8*). In patients with compromised renal function, gimeracil clearance is decreased, leading to high concentrations of 5-FU in blood and an increased risk of 5-FU-related side effects (*9*). Previously, we found that the incidence of grade 3 or higher diarrhea in metastatic colorectal cancer patients in the SOFT trial who were treated with SOX and bevacizumab depended on renal function (*5*). Thus, the incidence of diarrhea in patients with a creatinine clearance (Ccr) of < 70 mL/min before treatment exceeded 20% and tended to be higher than in patients with a Ccr of ≥ 70 mL/min.

It had been previously established that female patients treated with fluoropyrimidines developed leukopenia, stomatitis, diarrhea, nausea, vomiting, and alopecia more often and more severely than males (*10-15*). It was suggested that poorer clearance of 5-FU, reduced activity of dihydropyrimidine dehydrogenase (DPD; the initial enzyme in the catabolism of 5-FU (*10,16*)), and polymorphism of DPD or thymidylate synthase (*13,17*) are possible causes of such sex-related differences in adverse events during fluoropyrimidine treatment. However, the fundamental cause of this perceived sex difference is not yet known. DPD expression and activity in human liver did not reveal any sex-related differences (*13*). Dose modification and the administration schedule of 5-FU, and changing optimal supportive therapies for female patients, are not usually considered and are not implemented in clinical practice. Sex differences in the toxicity of anti-cancer agents are not only observed for 5-FU but also cisplatin, doxorubicin, and other anti-cancer agents (*18*). Female patients had significantly higher rates of nausea and vomiting, but the cause of the sex discrepancy is also unknown (*19*). We need to investigate any sex differences in disease and biological response in comparison of males and females genetically and epigenetically.

Several reports have documented a poor prognosis for advanced resectable colorectal cancer with poorly-differentiated adenocarcinoma histology relative to well- or moderately-differentiated adenocarcinoma. However, whether this also applies to unresectable metastatic cancer during palliative chemotherapy was not known (*20,21*). Poorly-differentiated adenocarcinoma is closely associated with the presence of microsatellite instability (MSI) and is found more often in females. Microsatellite unstable poorly-differentiated adenocarcinoma (23%, 12/53), which is characterized as having a right colon predilection, larger size, and infrequent lymph node metastasis, has a better prognosis than microsatellite-stable poorly-differentiated adenocarcinoma (74%, 41/53) (*22,23*).

In the present study, we aimed to evaluate and compare the safety of the SOX plus bevacizumab and FOLFOX plus bevacizumab regimens in female and male patients with metastatic colorectal cancer and the impact on histological tumor type treatment efficacy.

Patients and Methods

Patients

The SOFT trial was a randomized, open-label, phase III study that compared the efficacy and safety of the SOX/bevacizumab and FOLFOX/bevacizumab regimens in patients with unresectable advanced or recurrent metastatic colorectal cancer (*5*). SOX/bevacizumab was confirmed to be non-inferior to FOLFOX/bevacizumab in 512 randomized patients. In the SOX/bevacizumab regimen, S-1 was given orally for the first 2 weeks of a 3-week cycle, oxaliplatin at a dose of 130 mg/m^2 and bevacizumab at 7.5 mg/kg infused on day 1. In the FOLFOX/bevacizumab regimen, patients received a 5 mg/kg intravenous infusion of bevacizumab and a simultaneous intravenous infusion of 85 mg/m^2 oxaliplatin, 200 mg/m^2 *l*-leucovorin, 400 mg/m^2 bolus fluorouracil, and 2,400 mg/m^2 infused fluorouracil (46 h) delivered with an infusion pump on day 1 to 2 of a 2-week cycle. A 5-hydroxytryptamine-3 (5-HT$_3$) receptor antagonist and dexamethasone were usually given to patients treated with either SOX or FOLFOX. The Ccr was estimated using the Cockcroft-Gault equation.

Statistical analysis

We did analyses of survival by modified intention to treat: we excluded individuals who underwent randomization but who were subsequently shown not to meet inclusion criteria. Patients who received at least one dose of the assigned study drugs were included in analyses of safety.

The incidence of adverse events during the first 8 weeks and then all periods was compared between the two regimens using Fisher's exact test and logistic regression for males and females separately. Multivariate analyses for toxicities were also carried out using a logistic regression model. Adverse events were assessed in accordance with the Common Terminology Criteria for Adverse Events version 3.0. Median OS and PFS were estimated using the Kaplan-Meier method. Statistical significance was considered to be $P < 0.05$.

Multivariate analyses by Cox proportional hazards model was used to estimate hazard ratios (HRs) of prognostic factors for OS and PFS. Treatment delivery was evaluated for females and males in both treatment groups.

Statistical analyses were performed using SAS version 9.4 software (SAS Institute, Cary, NC).

Results

Baseline characteristics of all patients enrolled in the SOFT study were similar in the two sexes and in the treatment groups, although the proportion of patients with liver metastases in the SOX/bevacizumab group was higher in females than males. The proportion of female patients treated with SOX/bevacizumab and FOLFOX/bevacizumab was 33% (83/250) and 37% (93/249), respectively, for primary analysis (Table 1).

Safety

The most common hematologic adverse events of grade 3 or higher were leukopenia in 21 (8%) of 249 patients given FOLFOX/bevacizumab *vs*. 6 (2%) of 250 given SOX/bevacizumab ($P = 0.0029$) and neutropenia in 84 (34%) *vs*. 22 (9%) ($P < 0.0001$). Grade 3 or higher diarrhea in 23 (9%) *vs*. 7 (3%) ($P = 0.0040$) were significantly more common in patients given SOX/bevacizumab than in those given FOLFOX6/bevacizumab. Adverse events are shown in Table 2 (Appendix). Female patients treated with SOX/bevacizumab developed nausea and vomiting significantly more frequently than males, while females treated with FOLFOX/bevacizumab exhibited more leukopenia, neutropenia, nausea, vomiting, and alopecia 8 weeks after the beginning of each treatment cycle and over the entire treatment period. The difference between the sexes in the incidence of nausea and vomiting after FOLFOX/bevacizumab was more marked in patients with Ccr > 70 mL/min. According to multivariate analysis, sex was an independent predictive factor for nausea and vomiting due to SOX/bevacizumab, and for leukopenia, neutropenia, nausea, vomiting, and alopecia due to FOLFOX/

Table 1. Baseline characteristics of male and female patients

Items	SOX/Bev (n = 250)					FOLFOX/Bev (n = 249)				P
	Male (n = 167)		Female (n = 83)			Male (n = 156)		Female (n = 93)		
	n	%	n	%	P	n	%	n	%	
Age										
< 70	120	71.9	67	80.7	0.164	116	74.4	69	74.2	0.164
≥ 70	47	28.1	16	19.3		40	25.6	24	25.8	
Primary lesion										
Colon	80	47.9	48	57.8	0.422	76	48.7	47	50.5	0.422
Rectosigmoid	30	18.0	14	16.9		21	13.5	19	20.4	
Rectum	55	32.9	20	24.1		59	37.8	27	29.0	
Others	2	1.2	1	1.2		0		0		
Differentiation assessed by histology										
Well or moderate	148	88.6	67	80.7	0.223	135	86.5	77	82.8	0.223
Poorly	6	3.6	5	6.0		4	2.6	6	6.5	
Other	13	7.8	11	13.3		17	10.9	10	10.8	
Adjuvant chemotherapy for colorectal cancer										
No	142	85.0	72	86.7	0.849	128	82.0	83	89.2	0.849
Yes	25	15.0	11	13.3		28	18.0	10	10.8	
Target lesion										
No	12	7.2	9	10.8	0.340	11	7.0	11	11.8	0.340
Yes	155	92.8	74	89.2		145	93.0	82	88.2	
Liver metastases										
No	45	26.9	38	45.8	0.004	53	34.0	34	36.6	0.004
Yes	122	73.1	45	54.2		103	66.0	59	63.4	
Lung metastases										
No	93	55.7	50	60.2	0.501	87	55.8	47	50.5	0.501
Yes	74	44.3	33	39.8		69	44.2	46	49.5	
Lymph node metastases										
No	123	73.7	66	79.5	0.350	119	76.3	67	72.0	0.350
Yes	44	26.3	17	20.5		37	23.7	26	28.0	
Other metastases										
No	135	80.8	53	63.9	0.005	136	87.2	70	75.3	0.005
Yes	32	19.2	30	36.1		20	12.8	23	24.7	
Metastatic organs										
1	73	43.7	38	45.8	0.788	83	53.2	44	47.3	0.788
≥ 2	94	56.3	45	54.2		73	46.8	49	52.7	

P: Fisher's exact test. Bev, bevacizumab; FOLFOX, 5-FU/*l*-leucovorin plus oxaliplatin; SOX, S-1 plus oxaliplatin.

bevacizumab at 8 weeks and over all cycles (Table 3, Appendix). Thrombocytopenia with SOX/bevacizumab and FOLFOX/bevacizumab was more frequent in patients with Ccr < 70 mL/min and lower body mass index (BMI) after 8 weeks. Thrombocytopenia after FOLFOX/bevacizumab also developed more often in patients ≥ 70 years of age.

The mean relative dose intensities (RDIs) of S-1 during all cycles of SOX/bevacizumab were significantly lower in females (73.9%) than males (81.5%) ($P < 0.001$), while the RDIs of continuous infusion of 5-FU in FOLFOX/bevacizumab were 75.0% in females and 80.5% in males ($P = 0.005$) (Table 4, Appendix). The RDIs of oxaliplatin were not significantly different between female and male patients treated with either SOX/bevacizumab or FOLFOX/bevacizumab.

Efficacy

No significant differences in efficacy with regard to OS and PFS were identified between the sexes. The worse prognostic factor was poorly differentiated adenocarcinoma for OS ($P < 0.0001$) and PFS ($P = 0.0079$) (Table 5 and Table 6 (Appendix), Figure 1).

Discussion

Nausea and vomiting due to treatment with SOX/bevacizumab, and leukopenia, neutropenia, nausea, vomiting and alopecia due to FOLFOX/bevacizumab were more frequent in female patients than males in multivariate analysis. Sex differences in response to fluoropyrimidines and irinotecan combination therapy were also reported in a recent randomized trial, PETACC-3. These findings document a statistically significant and clinically relevant greater risk of nonhematological and objectively measurable hematological adverse events in female patients (*24*).

Nausea and vomiting are the most common adverse reactions associated with chemotherapy that can significantly diminish patient quality of life. To mitigate this, the use of 5-HT_3 receptor antagonists and dexamethasone have been recommended by the guidelines from the Japanese Society of Clinical Oncology (JSCO), the American Society of Clinical Oncology (ASCO), and the Multinational Association of Supportive Care in Cancer / European Society for Medical Oncology (MASCC/ESMO) (*25-27*). A Japanese phase III randomized controlled trial, the SENRI trial, was conducted in > 400 colorectal cancer patients treated with oxaliplatin-based chemotherapy. This trial established that a combination of 5-HT_3 receptor antagonists, dexamethasone and aprepitant/fosaprepitant was superior to the combination of 5-HT_3 receptor antagonists and dexamethasone alone in controlling nausea and vomiting over the entire treatment period, especially in the late phase (*28*). Other recent Japanese phase III trials have documented a

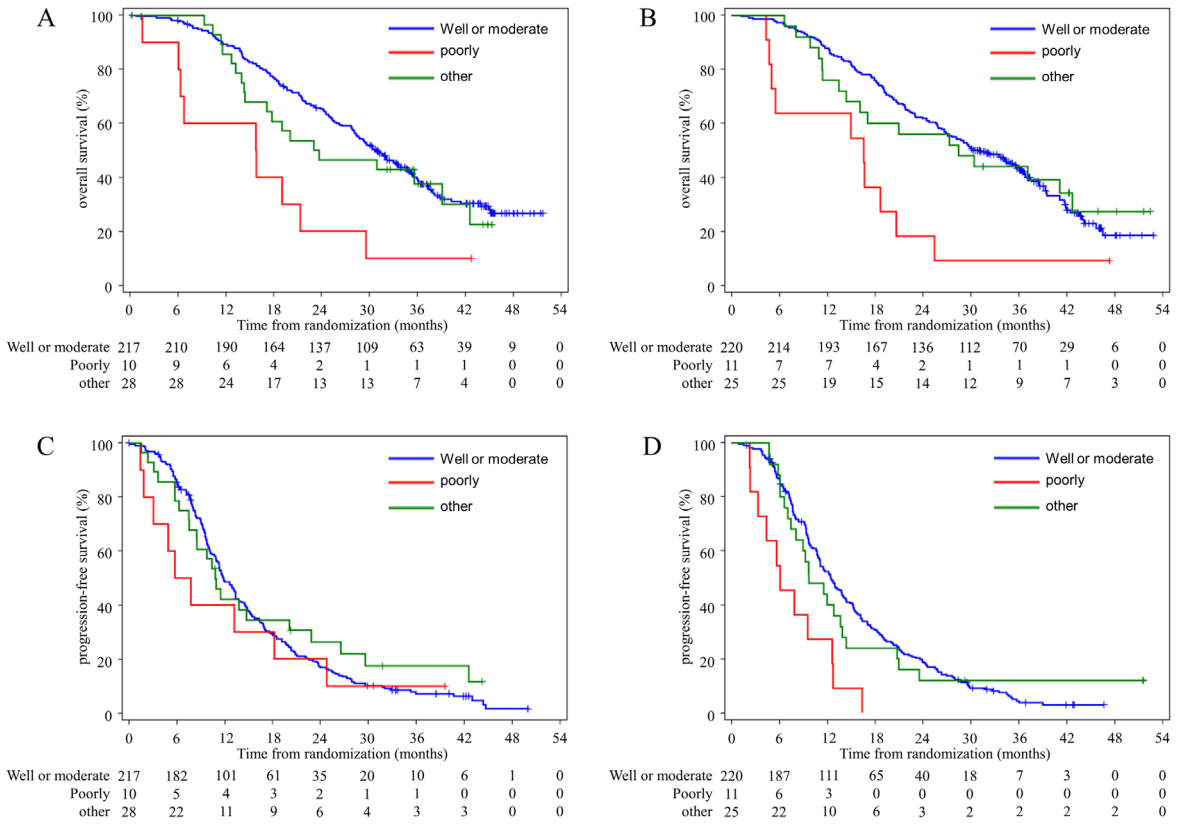

Figure 1. Kaplan-Meier estimates of overall survival according to histology of colorectal cancer treated with FOLFOX/Bev (A) or SOX/Bev (B) and progression-free survival with FOLFOX/Bev (C) and SOX/Bev (D) in the full dataset.

superior efficacy of dexamethasone on day 2 to 3, and olanzapine at a dose of 5 mg plus standard antiemetic therapy with 5-HT_3 receptor antagonists, aprepitant, and dexamethasone on day 1 (*29*). Female sex is a well-known risk factor for chemotherapy-induced nausea and vomiting, and we should therefore consider treatment options employing consecutive dexamethasone on day 2 to 3 and olanzapine for female patients receiving oxaliplatin-based regimens as is done for treatment with highly emetogenic chemotherapeutic agents (*25,30*).

Significantly more patients given FOLFOX/bevacizumab had grade 3 or higher leukopenia, neutropenia, and any grade of alopecia, than patients given SOX/bevacizumab, in a first analysis (*5*). In addition, females treated with FOLFOX/bevacizumab suffered leukopenia, neutropenia, and alopecia significantly more frequently than male patients. The proportions of patients with grade 3 or higher sensory neuropathy did not differ significantly between the groups. Ruzzo *et al.* recently reported that interactions of gene polymorphisms and sex on hematological toxicity of adjuvant therapy with FOLFOX or CapeOX were detected for MTHFR rs1801133 (*31*). In female patients, the ERCC1 rs11615 CC genotype worsened grade 3 or more neurological toxicity, as did XPD rs13181G, for example. Genomic effects have rarely been analyzed by sex, but such approaches may reveal sex differences in adverse events in the near future. Leukopenia and neutropenia occurred more often in patients ≥ 70 years of age, according to multivariate analysis. Decreased hematopoietic capacity and proliferation of monoclonal hematopoietic cells in the elderly may affect the higher incidence of leukopenia and neutropenia despite relatively mild SOX/bevacizumab effects on bone marrow suppression (*32*). Thrombocytopenia after SOX/bevacizumab was more frequent in patients with Ccr < 70 mL/min and lower BMI 8 weeks from the beginning of chemotherapy. Thrombocytopenia after FOLFOX/bevacizumab was also more common in patients with a lower Ccr, lower BMI, and aged ≥ 70 years. Cespedes Feliciano *et al.* reported that a higher proportion of patients in the lowest versus highest tertile of muscle mass experienced neutropenia (55% *vs.* 38%, $P = 0.008$) and thrombocytopenia (13% *vs.* 5%; $P = 0.02$) (*33*). Low muscle mass was associated with poor chemotherapy outcomes in that severe adverse events were more likely, either because patients with low muscle mass are over-dosed or because they are more frail or have an older functional age, conferring a higher risk of toxicity. However, no significant sex differences were observed in the incidence of subjective adverse reactions like stomatitis because patients could temporarily stop oral S-1 by themselves, depending on their symptoms, following their education in adequate self-administration routines. This is in contrast to infusions of 5-FU that patients cannot control themselves. The proportions of patients with grade 3 or greater diarrhea was significantly higher in the group given SOX/bevacizumab than in those given FOLFOX/bevacizumab, especially in patients with lower Ccr. On the other hand, there was no sex difference in the incidence of diarrhea.

A significantly more frequent incidence of nausea, vomiting, neutropenia, thrombocytopenia, and alopecia was seen with bolus 5-FU compared with its protracted venous infusion, similar to S-1 in terms of pharmacokinetics (*34,35*). 5-FU clearance is significantly lower in females than in males regardless of age and the dose given (*10*). Females receive supra-optimal doses compared with males (*36*) and higher plasma 5-FU concentrations are significantly related to more severe neutropenia and stomatitis (*37*). It would be difficult to explain the higher incidence of toxicities of 5-FU by rare DPYD variants. Only 3-5% of Caucasians have reduced DPD activity (*38-40*) and patients without a DPYD variant resulting in decreased or lack of function may still experience severe toxicity due to additional genetic, environmental, or other factors (*41,42*).

Although a significantly worse prognosis for poorly-differentiated adenocarcinoma was observed here, similar to previous reports (*20,21,43*), genomic analyses of somatic mutations or MSI were not carried out in the SOFT trial. Information on tumor location (right or left side) was also not recorded in this trial, other than whether the tumor was in the colon or rectum. Therefore, nothing can be said on this topic. Intensive anti-emetic therapy should be considered at least because of the higher incidence of nausea and vomiting in SOX/bevacizumab and FOLFOX/bevacizumab-treated female patients. It is difficult to argue for reducing the starting dose of SOX for female patients to compensate for the higher incidence of adverse events compared with males because severe toxicities are rarely induced by SOX. In conclusion, sex differences regarding adverse reactions during treatment with SOX/bevacizumab or FOLFOX/bevacizumab were confirmed in the SOFT study. This warrants further fundamental research to pursue the underlying cause.

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Conflict of interest

YY has received honoraria from Taiho, Chugai, Nipponkayaku, Japan. KM has received honoraria from Eli Lilly, Chugai, Takeda, Ono, Taiho, Sanofi, Bristol-Myers Squibb, and Bayer; and research funding from Parexel International, Merck Serono, Daiichi-Sankyo, Sumitomo-Dainippon Pharma, Shionogi, Pfizer, Mediscience Planning, and Solasia Pharma. HB has received honoraria from Taiho and Chugai; and research

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Table 2. Adverse events of any grade after 8 weeks of therapy and over the entire treatment period with S-1 plus oxaliplatin and bevacizumab or 5-FU/*l*-LV plus oxaliplatin and bevacizumab in patients stratified by creatinine clearance

	SOX/Bev (*n* = 250)							FOLFOX/Bev (*n* = 249)						
	Female (*n* = 83)		Male (*n* = 167)					Female (*n* = 93)		Male (*n* = 156)				
	n	%	*n*	%	Fisher *P*[(a)]	OR [95% CI]	*P*	*n*	%	*n*	%	Fisher *P*[(a)]	OR [95% CI]	*P*
8 weeks														
Leukopenia	30	36.1	47	28.1	0.244	1.45 [0.83-2.53]	0.198	63	67.7	70	44.9	<0.001	2.58 [1.51-4.41]	**<0.001**
CCr 70 mL/min >	9	50.0	12	40.0	0.558	1.50 [0.46-4.87]	0.500	22	75.9	13	44.8	0.031	3.87 [1.26-11.9]	**0.018**
70 mL/min≦	21	32.3	35	25.5	0.319	1.39 [0.73-2.65]	0.317	41	64.1	57	44.9	0.014	2.19 [1.18-4.07]	**0.013**
Neutropenia	26	31.3	50	29.9	0.884	1.07 [0.60-1.89]	0.823	61	65.6	63	40.4	<0.001	2.81 [1.65-4.80]	**<0.001**
CCr 70 mL/min >	10	55.6	10	33.3	0.147	2.50 [0.75-8.30]	0.135	18	62.1	11	37.9	0.114	2.68 [0.93-7.74]	**0.069**
70 mL/min≦	16	24.6	40	29.2	0.614	0.79 [0.40-1.55]	0.497	43	67.2	52	40.9	<0.001	2.95 [1.57-5.55]	**<0.001**
Thrombocytopenia	21	25.3	55	32.9	0.245	0.69 [0.38-1.25]	0.218	25	26.9	51	32.7	0.394	0.76 [0.43-1.34]	0.336
CCr 70 mL/min >	8	44.4	16	53.3	0.766	0.70 [0.22-2.27]	0.552	11	37.9	14	48.3	0.596	0.66 [0.23-1.86]	0.427
70 mL/min≦	13	20.0	39	28.5	0.230	0.63 [0.31-1.28]	0.201	14	21.9	37	29.1	0.305	0.68 [0.34-1.38]	0.286
Nausea	50	60.2	55	32.9	<0.001	3.09 [1.79-5.32]	**<0.001**	48	51.6	55	35.3	0.012	1.96 [1.16-3.30]	**0.012**
CCr 70 mL/min >	10	55.6	12	40.0	0.375	1.88 [0.58-6.12]	0.297	11	37.9	8	27.6	0.577	1.60 [0.53-4.85]	0.403
70 mL/min≦	40	61.5	43	31.4	<0.001	3.50 [1.89-6.48]	**<0.001**	37	57.8	47	37.0	0.009	2.33 [1.26-4.31]	**0.007**
Vomiting	19	22.9	14	8.4	0.003	3.24 [1.53-6.87]	**0.002**	18	19.4	11	7.1	0.007	3.16 [1.42-7.04]	**0.005**
CCr 70 mL/min >	7	38.9	4	13.3	0.074	4.14 [1.00-17.1]	**0.049**	5	17.2	1	3.4	0.194	5.83 [0.64-53.5]	0.119
70 mL/min≦	12	18.5	10	7.3	0.028	2.88 [1.17-7.06]	**0.021**	13	20.3	10	7.9	0.018	2.98 [1.23-7.25]	**0.016**
Diarrhea	31	37.3	56	33.5	0.575	1.18 [0.68-2.05]	0.551	27	29.0	29	18.6	0.061	1.79 [0.98-3.27]	0.058
CCr 70 mL/min >	12	66.7	15	50.0	0.369	2.00 [0.59-6.73]	0.263	9	31.0	5	17.2	0.358	2.16 [0.62-7.49]	0.225

	70 mL/min≦	19	29.2	41	29.9	1.000	0.97 [0.51-1.85]	0.920	18	28.1	24	18.9	0.195	1.68 [0.83-3.39]	0.148
Stomatitis		17	20.5	35	21.0	1.000	0.97 [0.51-1.86]	0.931	31	33.3	43	27.6	0.390	1.31 [0.75-2.29]	0.336
	CCr 70 mL/min >	4	22.2	3	10.0	0.400	2.57 [0.50-13.1]	0.256	9	31.0	12	41.4	0.585	0.64 [0.22-1.88]	0.414
	70 mL/min≦	13	20.0	32	23.4	0.718	0.82 [0.40-1.69]	0.593	22	34.4	31	24.4	0.172	1.62 [0.84-3.13]	0.148
Alopecia		3	3.6	1	0.6	0.108	6.23 [0.64-60.8]	0.116	28	30.1	14	9.0	<0.001	4.37 [2.16-8.85]	**<0.001**
	CCr 70 mL/min >	0	0	0	0	-	-	-	11	37.9	3	10.3	0.030	5.30 [1.29-21.7]	**0.021**
	70 mL/min≦	3	4.6	1	0.7	0.099	6.58 [0.67-64.5]	0.106	17	26.6	11	8.7	0.002	3.81 [1.66-8.75]	**0.002**
Sensory neuropathy		60	72.3	128	76.6	0.534	0.80 [0.44-1.45]	0.453	62	66.7	106	67.9	0.889	0.94 [0.55-1.63]	0.834
	CCr 70 mL/min >	14	77.8	22	73.3	1.000	1.27 [0.32-5.03]	0.731	20	69.0	18	62.1	0.783	1.36 [0.46-4.03]	0.581
	70 mL/min≦	46	70.8	106	77.4	0.383	0.71 [0.36-1.38]	0.311	42	65.6	88	69.3	0.625	0.85 [0.45-1.60]	0.263
All periods															
Leukopenia		49	59.0	96	57.5	0.892	1.07 [0.63-1.82]	0.815	76	81.7	99	63.5	0.003	2.57 [1.39-4.78]	0.003
	CCr 70 mL/min >	13	72.2	20	66.7	0.757	1.30 [0.36-4.68]	0.688	24	82.8	22	75.9	0.747	1.53 [0.42-5.52]	0.518
	70 mL/min≦	36	55.4	76	55.5	1.000	1.00 [0.55-1.80]	0.990	52	81.3	77	60.6	0.005	2.81 [1.37-5.79]	**0.005**
Neutropenia		50	60.2	98	58.7	0.892	1.07 [0.62-1.82]	0.814	76	81.7	104	66.7	0.013	2.24 [1.20-4.17]	**0.011**
	CCr 70 mL/min >	12	66.7	16	53.3	0.546	1.75 [0.52-5.89]	0.367	24	82.8	23	79.3	1.000	1.25 [0.34-4.68]	0.738
	70 mL/min≦	38	58.5	82	59.9	0.879	0.94 [0.52-1.72]	0.850	52	81.3	81	63.8	0.013	2.46 [1.19-5.08]	**0.015**
Thrombocytopenia		52	62.7	123	73.7	0.080	0.60 [0.34-1.05]	0.075	45	48.4	90	57.7	0.189	0.69 [0.41-1.15]	0.155
	CCr 70 mL/min >	12	66.7	23	76.7	0.513	0.61 [0.17-2.22]	0.452	16	55.2	21	72.4	0.274	0.47 [0.16-1.40]	0.175
	70 mL/min≦	40	61.5	100	73.0	0.106	0.59 [0.32-1.11]	0.101	29	45.3	69	54.3	0.284	0.70 [0.38-1.27]	0.240
Nausea		58	69.9	72	43.1	<0.001	3.06 [1.75-5.36]	**<0.001**	62	66.7	77	49.4	0.009	2.05 [1.20-3.50]	**0.008**
	CCr 70 mL/min >	12	66.7	14	46.7	0.237	2.29 [0.68-7.70]	0.182	16	55.2	13	44.8	0.600	1.52 [0.54-4.26]	0.432
	70 mL/min≦	46	70.8	58	42.3	<0.001	3.30 [1.75-6.21]	**<0.001**	46	71.9	64	50.4	0.005	2.52 [1.32-4.80]	**0.005**
Vomiting		28	33.7	23	13.8	<0.001	3.19 [1.69-6.00]	**<0.001**	29	31.2	21	13.5	0.001	2.91 [1.54-5.50]	**0.001**

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	CCr 70 mL/min >	8	44.4	5	16.7	0.049	4.00 [1.05-15.2]	**0.042**	10	34.5	3	10.3	0.056	4.56 [1.10-18.9]	**0.036**
	70 mL/min≦	20	30.8	18	13.1	0.004	2.94 [1.43-6.06]	**0.004**	19	29.7	18	14.2	0.019	2.56 [1.23-5.32]	**0.012**
Diarrhea		48	57.8	85	50.9	0.347	1.32 [0.78-2.25]	0.301	41	44.1	55	35.3	0.180	1.45 [0.86-2.45]	0.167
	CCr 70 mL/min >	14	77.8	16	53.3	0.127	3.06 [0.82-11.5]	0.097	13	44.8	10	34.5	0.592	1.54 [0.54-4.45]	0.422
	70 mL/min≦	34	52.3	69	50.4	0.881	1.08 [0.60-1.95]	0.797	28	43.8	45	35.4	0.274	1.42 [0.77-2.62]	0.265
Stomatitis		31	37.3	72	43.1	0.415	0.79 [0.46-1.35]	0.384	48	51.6	75	48.1	0.603	1.15 [0.69-1.93]	0.590
	CCr 70 mL/min >	5	27.8	8	26.7	1.000	1.06 [0.29-3.92]	0.933	13	44.8	16	55.2	0.600	0.66 [0.24-1.86]	0.432
	70 mL/min≦	26	40.0	64	46.7	0.449	0.76 [0.42-1.38]	0.370	35	54.7	59	46.5	0.289	1.39 [0.76-2.54]	0.284
Alopecia		6	7.2	9	5.4	0.579	1.37 [0.47-3.98]	0.565	36	38.7	25	16.0	<0.001	3.31 [1.82-6.02]	**<0.001**
	CCr 70 mL/min >	0	0	2	6.7	0.521	-	0.952	14	48.3	3	10.3	0.003	8.09 [2.00-32.8]	**0.003**
	70 mL/min≦	6	9.2	7	5.1	0.357	1.89 [0.61-5.86]	0.271	22	34.4	22	17.3	0.011	2.50 [1.25-4.99]	**0.009**
Sensory neuropathy		74	89.2	154	92.2	0.479	0.69 [0.28-1.70]	0.423	85	91.4	139	89.1	0.665	1.30 [0.54-3.14]	0.561
	CCr 70 mL/min >	16	88.9	26	86.7	1.000	1.23 [0.20-7.51]	0.822	27	93.1	27	93.1	1.000	1.00 [0.13-7.62]	1.000
	70 mL/min≦	58	89.2	128	93.4	0.403	0.58 [0.21-1.64]	0.306	58	90.6	112	88.2	0.807	1.30 [0.48-3.51]	0.612

[a] Fisher's exact test; comparing frequency of adverse events.

Bev, bevacizumab; CCr, creatinine clearance rate; FOLFOX, 5-FU/*l*-leucovorin plus oxaliplatin; OR, odds ratio; SOX, S-1 plus oxaliplatin; 95% CI, 95% confidence interval.

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Table 3. Multivariate analyses for adverse events after 8 weeks and all periods from the beginning of treatment with S-1 plus oxaliplatin with bevacizumab or 5-FU/*l*-LV plus oxaliplatin with bevacizumab

					Objective variables								
		Explanatory variables	Base category		Leukopenia	Neutropenia	Thrombo-cytopenia	Nausea	Vomiting	Diarrhea	Stomatitis	Alopecia	Sensory neuropathy
8 weeks	SOX/Bev	Sex	male	OR	1.54	1.15	0.62	2.88	3.04	1.18	0.95	5.94	0.72
		Female *vs.* male		95% CI	0.86-2.74	0.64-2.07	0.33-1.15	1.66-5.01	1.41-6.56	0.67-2.09	0.49-1.83	0.60-58.80	0.39-1.33
				P value	0.147	0.642	0.131	**< 0.001**	**0.005**	0.572	0.871	0.128	0.291
		CCr	70 mL/min >	OR	0.61	0.70	0.31	0.66	0.37	0.36	1.45	-	0.77
		70 mL/min ≦ *vs.* 70 mL/min >		95% CI	0.31-1.22	0.35-1.41	0.15-0.62	0.32-1.35	0.15-0.91	0.18-0.71	0.58-3.60	-	0.35-1.67
				P value	0.165	0.314	**0.001**	0.255	**0.031**	**0.003**	0.424	0.958	0.502
		BMI	median >	OR	0.96	1.01	1.93	1.24	1.37	0.79	1.00	2.55	1.57
		Median ≦ *vs.* median > per sex		95% CI	0.55-1.69	0.57-1.76	1.08-3.45	0.73-2.12	0.63-2.98	0.46-1.37	0.54-1.87	0.26-25.45	0.87-2.83
				P value	0.899	0.986	0.028	0.428	0.429	0.406	0.995	0.426	0.139
		Age	70 >	OR	1.92	2.36	1.25	0.49	0.82	1.14	0.61	-	0.56
		70 ≦ *vs.* 70 >		95% CI	1.02-3.61	1.26-4.42	0.65-2.41	0.25-0.95	0.32-2.08	0.60-2.16	0.27-1.37	-	0.29-1.09
				P value	**0.043**	**0.007**	0.497	**0.035**	0.670	0.686	0.226	0.954	0.09
	FOLFOX/Bev	Sex	male	OR	2.61	2.92	0.68	2.12	3.26	1.82	1.25	4.13	0.98
		Female *vs.* male		95% CI	1.51-4.51	1.69-5.02	0.38-1.24	1.24-3.64	1.45-7.35	0.99-3.36	0.71-2.20	2.02-8.43	0.56-1.71
				P value	**< 0.001**	**< 0.001**	0.212	**0.006**	**0.004**	0.055	0.437	**< 0.001**	0.945
		CCr	70 mL/min >	OR	0.89	1.21	0.46	1.72	1.63	0.96	0.62	0.70	1.06
		70mL/min ≦ *vs.* 70 mL/min >		95% CI	0.46-1.75	0.62-2.35	0.23-0.93	0.87-3.41	0.58-4.59	0.45-2.06	0.31-1.24	0.31-1.58	0.54-2.09

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				P value	0.739	0.580	**0.030**	0.121	0.355	0.914	0.176	0.391	0.868
		BMI	median >	OR	1.16	1.13	1.86	1.01	0.65	1.35	1.16	0.70	1.54
		Median ≦ *vs.*		95% CI	0.68-1.98	0.67-1.92	1.03-3.36	0.59-1.71	0.29-1.47	0.73-2.51	0.66-2.05	0.34-1.43	0.89-2.67
		median > per sex		*P* value	0.575	0.643	**0.039**	0.984	0.300	0.345	0.599	0.321	0.124
		Age	70 >	OR	1.65	1.17	2.04	0.77	1.07	1.20	0.78	0.94	1.23
		70 ≦ *vs.* 70 >		95% CI	0.88-3.09	0.63-2.18	1.08-3.83	0.41-1.45	0.41-2.80	0.59-2.44	0.40-1.52	0.41-2.17	0.64-2.37
				P value	0.120	0.614	**0.027**	0.426	0.897	0.621	0.466	0.888	0.531
all	SOX/Bev	Sex	male	OR	1.06	1.07	0.57	2.87	3.00	1.32	0.76	1.29	0.65
		female *vs*. male		95% CI	0.61-1.82	0.62-1.85	0.32-1.01	1.63-5.07	1.58-5.71	0.77-2.27	0.44-1.33	0.44-3.83	0.26-1.62
				P value	0.848	0.796	0.053	**< 0.001**	**< 0.001**	0.307	0.342	0.642	0.355
		CCr	70 mL/min >	OR	0.59	1.06	0.72	0.73	0.55	0.66	1.89	1.31	1.33
		70 mL/min ≦ *vs.*		95% CI	0.29-1.19	0.54-2.09	0.34-1.52	0.36-1.49	0.24-1.23	0.33-1.31	0.91-3.92	0.26-6.50	0.46-3.85
		70 mL/min >											
				P value	0.140	0.862	0.391	0.387	0.144	0.232	0.090	0.745	0.355
		BMI	median >	OR	1.11	0.94	1.25	1.20	0.99	0.88	1.55	2.06	1.18
		Median ≦ *vs.*		95% CI	0.66-1.86	0.56-1.56	0.72-2.18	0.71-2.03	0.51-1.89	0.53-1.47	0.92-2.61	0.67-6.32	0.48-2.89
		median > per sex											
				P value	0.693	0.800	0.429	0.507	0.964	0.626	0.098	0.205	0.724
		Age	70 >	OR	1.20	1.00	0.78	0.51	0.54	1.05	0.87	0.80	0.57
		70 ≦ *vs.* 70 >		95% CI	0.65-2.23	0.54-1.83	0.41-1.49	0.27-0.96	0.23-1.26	0.58-1.93	0.46-1.62	0.21-3.09	0.22-1.51
				P value	0.560	0.992	0.448	**0.036**	0.156	0.865	0.657	0.741	0.258
	FOLFOX/Bev	Sex	male	OR	2.55	2.23	0.66	2.16	2.92	1.45	1.15	3.29	1.21
		Female *vs.* male		95% CI	1.35-4.79	1.18-4.21	0.38-1.13	1.25-3.73	1.52-5.58	0.85-2.48	0.68-1.94	1.80-6.02	0.49-2.95
				P value	**0.004**	**0.014**	0.127	**0.006**	**0.001**	0.170	0.602	**< 0.001**	0.680

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	CCr	70 mL/min >	OR	0.76	0.64	0.60	1.45	0.77	0.84	0.86	0.96	0.54
	70mL/min ≦ *vs.* 70 mL/min >		95% CI	0.35-1.66	0.29-1.42	0.30-1.17	0.74-2.81	0.35-1.73	0.43-1.64	0.45-1.64	0.46-2.02	0.16-1.78
			P value	0.491	0.275	0.134	0.277	0.534	0.616	0.648	0.921	0.310
	BMI	median >	OR	1.03	1.39	1.73	0.77	1.24	1.34	1.24	0.92	0.87
	Median ≦ *vs.* median > per sex		95% CI	0.57-1.83	0.77-2.50	1.01-2.95	0.45-1.30	0.64-2.40	0.78-2.27	0.74-2.07	0.50-1.69	0.37-2.04
			P value	0.933	0.277	**0.045**	0.323	0.527	0.287	0.420	0.782	0.750
	Age	70 >	OR	2.08	1.78	2.24	0.61	0.51	0.75	0.77	1.30	0.60
	70 ≦ *vs.* 70 >		95% CI	0.99-4.35	0.85-3.74	1.18-4.24	0.33-1.12	0.22-1.20	0.40-1.41	0.42-1.41	0.64-2.63	0.23-1.53
			P value	0.052	0.127	**0.013**	0.112	0.122	0.367	0.397	0.464	0.280

The median BMI of female patients was 21.3 kg/m^2, and the BMI of males was 22.0 kg/m^2; -, not evaluable

Bev, bevacizumab; BMI, body mass index; CCr, creatinine clearance; CI, confidence interval; FOLFOX, 5-FU/*l*-leucovorin plus oxaliplatin; OR, odds ratio; SOX, S-1 plus oxaliplatin.

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Table 4. Total dose and relative dose intensity

		SOX/Bev (*n* = 250)				FOLFOX/Bev (*n* = 249)			
		Male (*n* = 167)	Female (*n* = 83)	*P*		Male (*n* = 156)	Female (*n* = 93)	*P*	
Bevacizumab	Median	90.6	85.7	0.166	a	83.3	83.4	0.466	a
	Range	0-100	0-100			0-100	36.7-100		
	Mean	85.2	81.6	0.134	b	78.9	79.4	0.832	b
	SD	16.3	20.4			19.1	14.6		
Oxaliplatin	Median	77.6	71.9	0.141	a	64.8	58.3	0.202	a
	Range	5.3-100	27.7-100			21.4-100	18.2-100		
	Mean	74.3	70.2	0.157	b	65.3	61.7	0.219	b
	SD	21.4	20.7			23.0	20.7		
l-leucovorin	Median					87.8	84.8	0.017	a
	Range					48.6-100	55.6-100		
	Mean					86.4	82.9	0.017	b
	SD					11.0	11.8		
5-FU, bolus	Median					82.8	70.0	0.001	a
	Range					36.8-100	34.8-100		
	Mean					77.8	69.9	< 0.001	b
	SD					17.6	17.7		
5-FU, ci	Median					84.1	74.9	0.005	a
	Range					44.4-100	41.7-100		
	Mean					80.5	75.0	0.005	b
	SD					14.9	15.0		
S-1	Median	82.2	76.0	0.003	a				
	Range	16.7-104.2	0-100						
	Mean	81.5	73.9	< 0.001	b				
	SD	14.9	19.9						

a: Wilcoxon rank sum test; b: *t* test.

ci, continuous infusion; FOLFOX/Bev, *l*-leucovorin, 5-fluorouracil, oxaliplatin, and bevacizumab; SOX/Bev, S-1, oxaliplatin, and bevacizumab.

Table 5. Prognostic factors for overall survival in patients treated with S-1 plus oxaliplatin with bevacizumab or 5-FU/*l*-LV plus oxaliplatin with bevacizumab

Variables		Base category	SOX/Bev			FOLOX/Bev			**All patients**		
			HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Sex	Female *vs*. male	Male	0.97	0.69-1.35	0.84	1.25	0.90-1.73	0.17	1.12	0.89-1.41	0.32
Age	70 ≦ *vs.* 70 >	70 >	1.14	0.80-1.62	0.47	0.95	0.64-1.38	0.77	1.06	0.82-1.36	0.66
Primary lesion	Rectosigmoid *vs.* colon	Colon	0.78	0.50-1.20	0.26	0.86	0.54-1.38	0.54	0.84	0.61-1.15	0.26
	Rectum *vs.* colon	Colon	0.96	0.66-1.40	0.85	0.95	0.67-1.35	0.78	0.97	0.75-1.25	0.82
Histology	Poorly *vs.* well/ moderate	Well/ moderate	2.61	1.32-5.15	0.0056	2.41	1.12-5.17	0.024	2.72	1.67-4.44	< 0.0001
	Others *vs.* well/ moderate	Well/ moderate	0.90	0.52-1.53	0.70	1.25	0.75-2.04	0.39	1.07	0.74-1.53	0.72
Adjuvant chemotherapy	Yes *vs.* No	No	0.97	0.61-1.53	0.89	0.73	0.45-1.18	0.20	0.83	0.59-1.15	0.25
Target lesion	Yes *vs.* No	No	1.22	0.65-2.25	0.53	1.7	0.83-3.44	0.15	1.40	0.88-2.22	0.15
Liver metastases	Yes *vs.* No	No	1.12	0.69-1.80	0.63	1.46	0.88-2.38	0.14	1.27	0.90-1.78	0.16
Lung metastases	Yes *vs.* No	No	0.98	0.63-1.50	0.91	1.48	0.88-2.46	0.13	1.21	0.88-1.66	0.23
Lymph node metastases	Yes *vs.* No	No	1.25	0.76-2.06	0.38	1.41	0.89-2.20	0.14	1.33	0.95-1.84	0.092
Other metastases	Yes *vs.* No	No	1.29	0.79-2.09	0.31	1.32	0.78-2.223	0.30	1.26	0.89-1.77	0.18
Metastatic organs	2 ≦ *vs.* 1	1	0.90	0.52-1.55	0.72	0.98	0.54-1.75	0.94	0.96	0.65-1.39	0.82
Treatment	SOX/Bev *vs.* FOLFOX/Bev	FOLFOX/Bev	-	-	-	-	-	-	1.02	0.82-1.26	0.88

FOLFOX/Bev, *l*-leucovorin, 5-fluorouracil, oxaliplatin, and bevacizumab; HR, hazard ratio; SOX/Bev, S-1, oxaliplatin, and bevacizumab; 95% CI, 95% confidence interval.

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Table 6. Prognostic factors for progression-free survival in patients treated with S-1 plus oxaliplatin with bevacizumab or 5-FU/*l*-LV plus oxaliplatin with bevacizumab

Variables		Base category	SOX/Bev			FOLFOX/Bev			All patients		
			HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Sex	Female *vs.* male	Male	0.90	0.68-1.20	0.49	1.09	0.82-1.44	0.56	0.97	0.79-1.18	0.72
Age	70 ≦ *vs.* 70 >	70 >	1.05	0.77-1.43	0.77	0.81	0.58-1.12	0.20	0.89	0.71-1.11	0.30
Primary lesion	Rectosigmoid *vs.* colon	Colon	0.85	0.58-1.24	0.40	0.94	0.63-1.38	0.74	0.89	0.68-1.16	0.40
	Rectum *vs.* colon	Colon	0.94	0.67-1.30	0.71	0.81	0.59-1.11	0.19	0.85	0.68-1.06	0.15
Histology	Poorly *vs.* well/ moderate	Well/ moderate	3.33	1.74-6.35	0.0003	1.30	0.63-2.68	0.48	1.89	1.18-3.02	0.0079
	Others *vs.* well/ moderate	Well/ moderate	1.07	0.66-1.71	0.78	0.87	0.55-1.37	0.55	0.91	0.65-1.25	0.55
Adjuvant chemotherapy	Yes *vs.* No	No	0.68	0.45-1.02	0.059	0.75	0.51-1.11	0.15	0.75	0.57-0.99	0.038
Target lesion	Yes *vs.* No	No	0.85	0.51-1.40	0.52	1.17	0.70-1.95	0.58	0.96	0.67-1.37	0.83
Liver metastases	Yes *vs.* No	No	1.05	0.69-1.59	0.81	0.97	0.62-1.50	0.88	1.03	0.77-1.39	0.82
Lung metastases	Yes *vs.* No	No	1.19	0.82-1.72	0.35	1.26	0.82-1.94	0.29	1.24	0.94-1.62	0.12
Lymph node metastases	Yes *vs.* No	No	1.00	0.63-1.58	0.99	0.87	0.58-1.32	0.52	0.98	0.73-1.30	0.87
Other metastases	Yes *vs.* No	No	0.99	0.64-1.52	0.95	1.02	0.64-1.63	0.92	1.04	0.76-1.42	0.80
Metastatic organs	2 ≦ *vs.* 1	1	1.08	0.67-1.74	0.74	0.99	0.61-1.60	0.96	1.00	0.72-1.37	0.98
Treatment	SOX/Bev *vs.* FOLFOX/Bev	FOLFOX/Bev	-	-	-	-	-	-	1.06	0.88-1.28	0.51

FOLFOX/Bev, *l*-leucovorin, 5-fluorouracil, oxaliplatin, and bevacizumab; HR, hazard ratio; SOX/Bev, S-1, oxaliplatin, and bevacizumab; 95% CI, 95% confidence interval.

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BRIEF REPORT
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Assessing the feasibility of introducing an electronic health information system into Tuberculosis clinics and laboratories in Myanmar

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Abstract: Myanmar has launched an advanced tuberculosis examination policy, which involves specimen exchanges among clinics and referral laboratories. However, with the current paper-based operation, it is difficult to trace information accurately. Therefore, since April 2017, we introduced a pilot operation consisting of an electronic health information system (HIS) that uses QR codes for data sharing in the tuberculosis laboratory at seven facilities. This study aimed to assess the feasibility of introducing the electronic HIS into tuberculosis clinics and laboratories based on staff perception, workload and workflow, and data accuracy, and to clarify its advantages and disadvantages. The analysis was descriptive, and it involved a semi-structured interview for the staff, workflow observations to evaluate the workload and describe the change in workflow, and evaluation of the data accuracy by comparing the numbers yielded by the paper-based and HIS-based reports. The HIS was positively accepted as it improved work efficiency, while the operation still depended on paper-based reports. Parallel data registration using both paper-based and HIS-based reports increased the workload. Data discrepancies were found when comparing the paper-based and HIS-based reports, and these discrepancies were not directly attributed to the HIS introduction but individual factors. Crucial facilitating factors of the HIS were its operability and user-friendliness, because it does not require specific training. The additional workload translates into the need for additional human resources, and the parallel data registration remains a challenge. However, we consider that these challenges could be overcome as coverage of the HIS expands.

***Keywords*:** health information system, QR code, data sharing, tuberculosis, Myanmar

Introduction

Since the early 2000s, the increasing use of information and communication technologies (ICT) in health services in both developed and developing countries has resulted in the progressive development of eHealth. With this trend, many developing countries have attempted to introduce electronic health information systems (HIS) (*1*). In the era of Sustainable Development Goals, the need for introducing ICT for data management continues to grow (*2*).

Tuberculosis (TB) is one of the health priorities in Myanmar as this country appears in the three high burden country lists by the World Health Organization (WHO): TB, TB/HIV, and multi-drug resistant (MDR)-TB (*3*). The epidemic of MDR-TB has changed the control measures of TB as well as the diagnostic flow. Previously, for smear-positive cases, treatment was started at the nearest TB facility at which smear examination was available. Currently, all smear-positive cases should be screened for MDR-TB by GeneXpert® that is only available at selected sites. Patients diagnosed as MDR-TB by GeneXpert® should be monitored regularly by culture and drug sensitivity test (DST) and line probe assay (LPA) that are only available at National Reference Laboratories (*4,5*) (Figure S1, *https://www.globalhealthmedicine.com/site/supplementaldata.html?ID=8*). Therefore, there is frequent transfer of specimens with patient information during the diagnosis and treatment of TB. However, it is difficult to secure the traceability of specimens and patient information among clinics and laboratories by using the current paper-based operation. Thus, it was hypothesized that implementing a HIS to assist the National TB Program (NTP) of Myanmar could help improve TB patient data management, including traceability. Thus, the Myanmar Ministry of Health and Sports has launched the Strategic Action Plan for Strengthening Health Information 2017-2021 (*6*).

To contribute to the NTP, we developed the electronic TB laboratory HIS. The system is based on the use of a two-dimensional barcode (QR code) used to exchange and synchronize information, and an internet

connection is not required to operate the system. This system intends to reduce the workload of the staff who handle the patient data in clinics and laboratories while improving patient traceability. The registration of patient information is done using a touchscreen tablet device, which is aligned with the current paper-based format. At each visit, the information, such as name, sex, date of birth, and address, is inputted to the tablet, and patient information is outputted to the examination order sheet with a QR code. This examination order sheet is sent to a referral laboratory with the patient's specimens. At the laboratory, the QR code is scanned, and the patient's data and examination orders are registered into the system. The results of the laboratory tests are registered into the system, and the result sheet is printed out with the QR code, which goes back to the clinic. When the QR code is scanned at the clinic, the results of the laboratory tests are added automatically.

In April 2017, we introduced the HIS as a pilot operation at one National Reference Laboratory (NRL), two Regional Reference Clinics and Laboratories (RRCL), and two Township Clinics and Laboratories (TCL) in Yangon in cooperation with NTP. The pilot was also expanded to two additional TCLs in March 2018 (Table 1). This study aimed to assess the feasibility of introducing the electronic HIS into TB clinics and laboratories in Myanmar according to staff's perception, workload and workflow, and data accuracy. This study also aimed to clarify the advantages and disadvantages of HIS introduction.

Materials and Methods

The study was approved by the ethical committees of the National Center for Global Health and Medicine and School of Tropical Medicine and Global Health, Nagasaki University. The feasibility was assessed descriptively by semi-structured interviews, workflow observation, and the comparison of the reported patient number on the quarterly reports between the paper-based and the HIS-based.

Semi-structured interview

Semi-structured interviews were conducted on seven individuals between July 2017 and January 2018 as these were third and ninth months of the pilot system operation at the NRL, two RRCLs, and two TCLs. The number of interviewees was limited to seven since the assigned personnel in charge of HIS operation at each facility were two or one. The purpose of the interviews was to clarify the perception of the staff and identify achievements and challenges. We prepared an interview guide based on a review of relevant literature that included three categories: System operation, Challenges, and Expectations. We categorized the obtained qualitative data along with the three categories and summarized them.

Workflow observations

Workflow observations were conducted at one RRCL and four TCLs during July 2018. We identified and described the changes in workflows and evaluated the workload resulting from the paper-based and the HIS-based operations. We excluded the NRL as this operated differently from other facilities. Observations were described on who, where, when, and how the systems were used with the current paper-based operation using the prepared workflow-check sheet.

Evaluation for data accuracy

The data for the second and the third quarter at the four TCLs were collected during December 2018. The NRL and two RRCLs were excluded since they did not produce the quarterly reports. We referred to the registration book (TB-03 form) manually written by staff from individual outpatient-department (OPD) books and counted the numbers by type of patient and type of disease, in accordance with the classification used in the quarterly report. The counted numbers were regarded as the Accuracy test dataset, which was compared with the numbers on the HIS-produced quarterly reports and the manual quarterly reports (Figure 1).

Results and Discussion

Perception of the staff

Table 2 shows the summary of comments from semi-structured interviews. Overall, there were no specific

Table 1. System installation facilities

	Type of facility	Type of lab. exam. implemented*	Installation date
1	National reference laboratory (NRL)	Smear, GeneXpert, DST, LPA, Culture	April 2017
2	Regional reference clinic and lab. (RRCL-A)	Smear, GeneXpert	April 2017
3	Regional reference clinic and lab. (RRCL-B)	Smear, GeneXpert	April 2017
4	Township clinic and lab. (TCL-A)	Smear, GeneXpert	April 2017
5	Township clinic and lab. (TCL-B)	Smear	April 2017
6	Township clinic and lab. (TCL-C)	Smear	March 2018
7	Township clinic and lab. (TCL-D)	Smear, GeneXpert	March 2018

*Smear, smear microscopy; DST, drug sensitivity test; LPA, line prove assay.

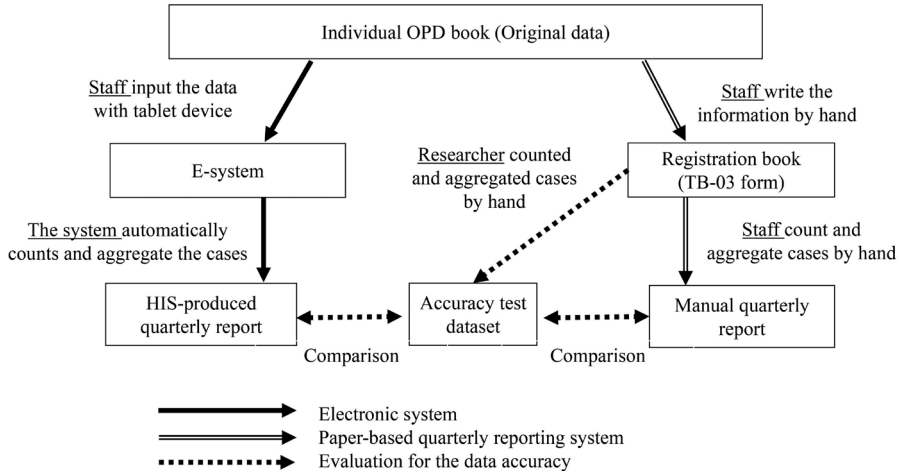

Figure 1. Method of the Evaluation for the data accuracy.

Table 2. Summary of comments from semi-structured interviews

Categories	Questions	Summary of comments
System operation	How long do you need operational support for system introduction?	Less than 1 month and the operation is not complicated.
	Do you still use an existing paper-based registration with the system operation?	Yes, system operation only is not permitted yet; the paper-based operation is more accurate in the workflow.
	Is the touch screen operation smooth and effective for data entry?	Easy to understand, but using the keyboard and mouse is preferable.
	Is patient search function easy and effective?	Easy, but need to add different ID numbers such as OPD, TB, and presumptive TB.
	Did QR code operation reduce the workload of data registration?	It reduces the time for data registration, but QR code sharing is limited still among facilities.
	When do you register patient info./ lab. test results into the system?	In the evening after registering on the paper book because of the high number of patients who visit at the same time and waiting for lab results.
		It is timely done in the workflow.
	Are there any changes to the workflow?	The system operation is extra work for now; need data entry into the system after paper registration.
	Has work efficiency improved by the system function?	Patient search is easy, but the function should be improved.
		QR code reduces the time for data exchange, but errors sometimes occur while scanning QR code
		The operation of the treatment card remains paper-based.
		The quarterly report still depends on the paper registration book.
	Is there any duplication of the patient registry?	Yes, when it's confusing to search for patients, they are registered as new cases.
		Data mis-entry occurs.
Challenges	What is the current problem with the system or work?	Parallel operation with paper-based is time-consuming.
		HR is needed for data entry.
		Patient search conditions should be improved.
		Errors occur during QR code reading and slow loading.
		During a blackout, the printer does not work.
Expectations	What kind of support do you expect?	Expansion of the system; QR code should be shared with other facilities so that the workload is further reduced.
		Provision of paper and printer toners.
		HR may be needed for the successful installation of other facilities.
		Adding other specific formats aligning with the paper operation.

differences of the perception between the NRL, two RRCLs, and two TCLs for introducing the HIS.

After the HIS introduction, the staff had a positive perception of the effectiveness of the QR code operation, although challenges remained. The paper operation was perceived as more accurate than the HIS. Additionally, both the paper-based and the HIS-based operations were being conducted simultaneously, which was time-consuming. The five respondents mentioned that patient registration in the HIS was done collectively at the end of the day because of the high number of patients, and doing both registrations simultaneously consumed time. Two respondents indicated that the HIS registration could be done in a timely manner within the regular workflow. None used the monthly reporting function on the HIS but still depended on the paper-based reporting. Additionally, a need for more human resources (HR) to enter data was reported. Advantages were noted by the five respondents: work efficiency was improved for patient searching and patient data and laboratory test results registration using the QR code. All respondents expected application of the HIS to help other facilities, because the workload could eventually be reduced by using the QR code. These findings indicate that the HIS operation was accepted with the expected operational efficiency, and users considered its expansion to other centers and widespread use of the QR code to be advantageous. Indeed, the QR code operation was successfully adopted without changing the main workflows and contributed to the instant data exchange (Table 3). Further, it can be expected that the operability of the system would further contribute to the positive perception of the staff as it does not require specific training. However, based on previous experiences, providing enough training is one of the essential elements for a successful introduction of an electronic HIS (*7-13*).

Impact on the workload and workflow

Data exchange between clinics and laboratories was implemented as intended using the QR code produced by the system at the OPD reception and laboratories to reduce the workload and improve work efficiency (Table 3). It was expected that parallel data registration with the paper-based and the HIS-based procedures at the OPD reception and laboratories is unavoidable during the introduction phase of the HIS as previously reported (*7,9,10,13-18*). Additionally, it was expected that the parallel data registration would help evaluate feasibility of the operation, as reported for the introduction of an electronic TB surveillance system (*9,19*). Considering the HR constraints tend to be the greatest challenge for the introduction of a HIS, the expansion of the QR code operation into other facilities would likely help reduce the workload for data registration. In TCLs, where the number of patients is relatively lower, HIS users were able to update the data without hiring data processing clerks. This indicates that the HIS does not necessarily require additional HR. We consider that any additional workload would be compensated by the benefit of having centralized patient information that was usually managed using different registration books, for example, TB patient, presumptive TB patient, laboratory examination, and OPD with different IDs. Because the treatment of TB patients is long term, the HIS will facilitate data transfers and exchanges using the patient identifier (QR code), which is crucial to monitor and trace the records effectively (*19*).

In terms of workflows, it was indicated that the HIS could be used simultaneously with the current paper-based operation without affecting the workflows. It was considered that the system interface aligned to the paper format helped the staff operate the HIS efficiently without training. Indeed, the operation of the laboratory-order sheets and the result sheets using the QR code produced by the systems have replaced the paper operation at the OPD receptions and the laboratories. Additionally, the system can work as an operational tool for handling case-based data for TB patients at the facility level. As the QR code contains all the necessary information for patients, it can be expected to improve the accuracy of data transfer as it has been previously reported for clinical paper-based and electronic sources (*20*).

The accuracy of data registration

Table 4 shows the reported numbers for the type of patient and type of disease for the second and the third quarters of 2018 at four TCLs (shown as TCL-A, B, C, and D). Each number in the HIS-based (HIS-produced quarterly report) and the Paper-based (manually quarterly report) were compared with the Accurate (Accuracy test dataset). The evaluation results indicated that accuracy most likely corresponded with personnel understanding but was not directly affected by the HIS introduction. In other words, there was no difference in the accuracy of data registration between HIS-based and Paper-based. It also indicated that the HIS was understandable and operable at each facility.

TCL-A, B, and C reported the number of patients completely accurately in the Paper-based, and we interpreted that the data registration into the HIS was nearly correct since the number of discrepancies found in the HIS-based was 1 to 3 due to incorrect data entry and automatic calculation by the system. Conversely, TCL-D incorrectly reported the number of patients with 25 to 63 discrepancies in both Paper-based and HIS-based, respectively. This was attributable to misinterpretations of patients' classification between Bacteriologically-confirmed cases and clinically diagnosed cases on the registration book (TB-03 form), which was based on the first result of the smear examination, positive or negative with symptoms. Accordingly, there is room for

Table 3. Findings from workflow observation

Site	Clinical work	Paper-based	HIS-based	Findings
Reception	· Registration	· OPD registration book · OPD card for each patient		
OPD/TB clinic	· Seen by doctor · Refer patients with TB symptoms to TB clinics · Order diagnostic test (sputum/Xp)	· Presumptive TB registration book (Note)	· Registration as presumptive TB · Printing out a Lab. request form with QR code	· Registration is not shifted to the HIS but parallelly operated with paper-based. · Lab. request form with QR code is functioned as intended. · The HIS is not operated directly at the doctor's consultation.
Laboratory	· Smear exam · Xpert for smear positive specimens (send the specimen to Xpert site for screening RR) · Culture for RR in Xpert	· Laboratory registration book	· Scanning QR code · Entering the Lab. results · Printing out a Lab. result form with QR code	· Registration is parallelly operated. · Data transfer by QR code is functioned.
TB clinic	· Register patients when diagnosed with drug-susceptive TB and start the treatment · Refer MDR patients to an MDR-TB clinic for treatment · Monitor treatment efficacy	· TB registration book (TB-03) · Treatment card (patient referral form)	· Scanning QR code · Registering as a TB case · Printing out a Lab. request form with QR code	· Registration is parallelly operated. · Data transfer by QR code is functioned. · Treatment card operation on the HIS is not implemented, but the paper-based is used.
Laboratory	· Smear exam (at least 3 times for 6-month treatment course)	· Laboratory registration book	· Scanning QR code · Entering the Lab. results · Printing out a Lab. result form with QR code	
TB clinic	· Evaluate treatment outcome · Reporting cases on a quarterly basis	· Quarterly report (TB-07, TB-08)		· The quarterly report is not produced by the HIS, but the paper-based is used as the main operation.

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Table 4. Accuracy of the reported number of patients on the HIS-based and Paper-based quarterly reports

Site	Quarter	Method	Type of patient				Total	Type of disease*				Total	Cause of discrepancy
			New	Re-treatment				Pul., Bac. confirmed	Pul., Cli. diagnosed	Ex-Plu., Bac. confirmed	Ex-Plu., Cli., diagnosed		
				Relapse	Prev. treated	Unknown							
A	2Q	Accurate	104	18	1	0	123	60	43	0	20	123	Data mis-entry
		HIS-based	104	18	1	0	123	60	43	0	20	123	
		Paper-based	104	18	1	0	123	60	43	0	20	123	
	3Q	Accurate	96	13	0	0	109	49	51	0	9	109	
		HIS-based	97 (+1)	12 (-1)	0	0	109	49	51	0	9	109	
		Paper-based	96	13	0	0	109	49	51	0	9	109	
B	2Q	Accurate	74	14	1	0	89	39	40	0	10	89	Data mis-entry, misclassification by the HIS
		HIS-based	75 (+1)	13 (-1)	0 (-1)	1 (+1)	89	39	41 (+1)	0	9 (-1)	89	
		Paper-based	74	14	1	0	89	39	40	0	10	89	
	3Q	Accurate	102	13	4	0	119	55	46	0	18	119	
		HIS-based	100 (-2)	14 (+1)	2 (-2)	2 (+2)	118 (-1)	54 (-1)	49 (+3)	0	15 (-3)	118 (-1)	
		Paper-based	102	13	4	0	119	55	46	0	18	119	
C	2Q	Accurate	139	32	0	0	171	95	63	0	13	171	Data mis-entry, misclassification by the HIS
		HIS-based	139	32	0	0	171	95	62 (-1)	1 (+1)	13	171	
		Paper-based	139	32	0	0	171	95	63	0	13	171	
	3Q	Accurate	135	18	2	0	155	84	62	0	9	155	
		HIS-based	135	18	0 (-2)	2 (+2)	155	83 (-1)	63 (+1)	0	9	155	
		Paper-based	135	18	2	0	155	84	62	0	9	155	
D	2Q	Accurate	280	33	4	0	317	178	108	0	31	317	Misunderstanding of the classification criteria, data mis-entry, misclassification by the HIS
		HIS-based	280	33	4	0	317	148 (-30)	140 (+32)	2 (+2)	26 (-5)	316 (-1)	
		Paper-based	280	33	4	0	317	174 (-4)	133 (+25)	0	10 (-21)	317	
	3Q	Accurate	329	37	3	0	369	178	157	0	34	369	
		HIS-based	329	37	3	0	369	115 (-63)	216 (+59)	1 (+1)	33 (-1)	365 (-4)	
		Paper-based	329	37	3	0	369	173 (-5)	162 (+5)	0	34	369	

Each "+," "-" and the following number shows the discrepancy of the number compared with the Accurate. *Pul, pulmonary; Bac, bacteriologically; Cli, clinically; Ex-Pul, extrapulmonary.

improvement of accuracy as these discrepancies were easily corrected once we intervened and gave necessary instructions. Indeed, we corrected the registered data in the HIS with the personnel and an administrator at TCL-D with the discussion of the NTP's criteria of patient classification.

Improvements of timeliness and completeness of reporting in the quarterly report were also expected once the cause of discrepancy was identified and corrected since the HIS can produce a report within a few seconds, while the paper operation may take up to a few days to generate a report. Patient numbers between the paper-based and HIS are then compared. This is one of the indicators that the criteria are in use (*19,20*).

Advantages and disadvantages of the HIS introduction

In this study, an advantage of the HIS was its user-friendliness. Additionally, it allowed alignment with the current paper-based operation. Other benefits are that the HIS targets resource-limited situations, and specific training and additional HR are not needed, which makes this a feasible operation. While the parallel data registration increased the workload and possibly hindered the acceptance of the staff or administrators, we expect further effective operation including several aspects such as interoperability, patient traceability, and patient registry.

The interoperability of the QR code may be another advantage as the introduction of electronic HIS should be integrated for avoiding complexity among other HIS (*7,9,10,16-18,21*). In Myanmar, MDR-TB patient information is managed by OpenMRS, which is one of the HIS adopted by NTP and was installed at the NRL (*5*). We discussed this with a team from OpenMRS, adopting the system possible to share data using the QR code among both HIS. Understanding the context of the country and the region is crucial such as the national guideline and the operational flows at the facility level.

Improvement of patient traceability is also expected because of the QR code. In the current networking of TB patients, information and laboratory specimens were all moving in different directions; thus, it takes manual data registration using the different types of registration books at each facility. This situation makes tracing patients challenging, and it has sometimes caused missing patients. Therefore, the QR code starts a battery specimen, which would be a strong point in terms of patient traceability.

As the internet infrastructure is rapidly growing, the tools assessed herein may need to be updated for future utility in Myanmar. Considering these aspects, the system is designed to allow for internet-based data storage once the environment is secured in the future. We presume that data sharing using QR code is the first step for the digitalization of the HIS. Furthermore, it would contribute to establishment of a client registry with the integration of aggregated health information according to the national strategy in Myanmar (*6*).

Feasibility of the HIS introduction

In conclusion, this study showed that indeed it is feasible to introduce an electronic HIS intended to align with the current paper-based format and adopting QR code operation in TB clinics and laboratories in Myanmar. The user-friendliness, no need for specific training, or additional HR are the main advantages of the HIS introduction in a resource-limited situation. The additional workload of the parallel data registration may require additional HR and remains a challenge; however, it would be expected to overcome this challenge as the use of the HIS expands and there is interoperability with other HIS. The system operation is still in the pilot phase and not fully operational or widespread. Thus, we were unable to show improvement in operational outcomes such as the timeliness and completeness for reporting, interoperability with other HIS, and patient traceability. Further research and follow-up for the system expansion would make it possible to show more convincing outcomes to attract staff, administrators, and policymakers.

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Screening for dental focal infections in febrile patients with hematologic malignancies who received chemotherapy: a retrospective cohort study

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Abstract: Source of fever in chemotherapy patients is often unknown. Fever can also be fatal. No observational studies have determined the incidence of dental focal infection (DFI)-associated fever, despite oral cavity being a potential source of infection. We report the incidence of fever after chemotherapy in patients with hematological malignancies and their association with DFIs in 441 patients visiting our institution during a 6-year period. Dental treatments, including tooth extraction, were performed, and their oral and hematological profiles were monitored after chemotherapy. Fever was evident in 87 (38.5%) of 226 patients (≥ 38˚C) after the first cycle of chemotherapy. Sepsis due to DFIs (n = 4; 4.6%) was evaluated. Chemotherapy was delayed due to DFI in one case. Fever after chemotherapy should be differentiated from oral infections. Our study emphasizes the significance of DFI in patients with fever after chemotherapy and can help in improving the prognosis of patients.

***Keywords*:** fever, hematopoietic neoplasm, immunosuppression, infection, oral cavity

Patients with hematologic malignancy are susceptible to infections due to myelosuppression during their chemotherapy regimen (*1,2*). Fever is a frequent adverse event associated with chemotherapy and often represent the only clinically evident sign of a serious infection in these immunocompromised patients (*3*). Although early identification of the etiology of fever and administration of appropriate treatment are essential, because fever can sometimes lead to death even without a clear cause of fever (*4*), particularly in patients with myelosuppression. However, establishing a precise and early diagnosis is challenging (*5,6*). The oral cavity is a potential site for infection, and dental focal infections (DFIs), such as periodontitis and pericoronitis, have been reported to cause life-threatening systemic morbidities (*7,8*). However, no observational study has adequately followed and investigated the incidence of DFI-associated fever and potential oral factors that affect the chemotherapy schedule in patients with hematologic malignancies.

We conducted a study to examine the incidence of fever after chemotherapy and emphasize the importance of intraoral evaluation in patients with hematologic malignancies who need chemotherapy. This is a retrospective cohort study using medical records obtained from the Department of Oral and Maxillofacial Surgery of our institution. We selected patients with hematologic malignancies, such as multiple myeloma, malignant lymphoma, myeloid/lymphoid leukemia, and myelodysplastic syndrome, who received chemotherapy at the Department of Hematology of our institution from January 2011 to December 2016. To avoid duplication of target patients, we excluded patients who received chemotherapy before the study period. In the included patients, we investigated the incidence of fever; predicted sources of fever, including DFIs that were reported in their medical records; and their adverse impact on chemotherapy schedule. Additionally, we examined the duration from fever onset to the diagnosis of the etiology of fever and analyzed the differences using regression analysis. The observation period for each patient was 30 days from the initiation of first cycle of antineoplastic chemotherapy. Patients with inadequate follow-up were excluded. In the present study, fever was defined by an axillary temperature of ≥ 38˚C.

In total, 441 patients with hematologic malignancies visited our institution during the study period. After

exclusion, we identified 226 patients (133 men, 58.8%; 93 women, 41.2%) with a median age of 65 (range, 16-93) years at first visit to our department. All patients received oral health care and appropriate dental treatments, including tooth extraction (n = 82, 36.3%), before chemotherapy. The most common hematologic diagnosis was malignant lymphoma (n = 116, 51.3%), followed by multiple myeloma (n = 53, 23.5%), myeloid/lymphoid leukemia (n = 41, 18.1%), and myelodysplastic syndrome (n = 16, 7.1%). Among these patients, 87 (38.5%) experienced fever (≥ 38˚C) after chemotherapy (Table 1), including fever of unknown origin (FUO) (n = 19, 21.8%), febrile neutropenia without evidence of infection (FN) (n = 17, 19.5%), tumor-related fever (n = 17, 19.5%), drug-related fever (n = 9, 10.3%), and systemic bacterial or viral infection (n = 25, 28.7%). The most frequently observed causes of systemic infection were catheter-related blood stream infection and pneumonia (n = 6, 6.9%). Sepsis due to DFIs (n = 4, 4.6%), such as pericoronitis of the wisdom tooth (n = 1), acute marginal periodontitis (n = 1), and surgical site infection (SSI) associated with tooth extraction (n = 2), were also observed (Table 2). The difference in the time taken to diagnose infection-associated fevers could not be analyzed due to the small sample size; in three of the four DFI-associated patients who experienced fever, approximately two weeks were needed to diagnose the cause of fever (1, 13, 15, and 27 days, respectively). Furthermore, in one patient, chemotherapy was postponed because of SSI associated with tooth extraction. No significant difference was noted in the number of patients with fever who underwent tooth extraction and those who did not (58 cases, 66.7%, and 29 cases, 33.3%, respectively; p = 0.48 by two-tailed t-test).

Although the importance of screening for infection before initiating chemotherapy has long been recognized, dental-medical cooperation and sharing of relevant information between dental and medical staff can be improved further (*7*). Our hospital follows a clinical protocol to identify potential sources of infection in the stomatognathic region in patients with hematologic malignancy scheduled to receive their first cycle of chemotherapy. This protocol enabled us to record and track the incidences of oral adverse events after chemotherapy; this benefit indicates that medical cooperation between the departments of dentistry and hematology is extremely valuable to improve the treatment outcome of patients with hematologic malignancies.

We report that fever (≥ 38˚C) occurred within 30 days from the first cycle of antineoplastic chemotherapy

Table 1. Distribution of hematologic diagnosis and febrile patients after chemotherapy

Hematologic diagnosis	Male	Female	Total	No. of patients with fever	95% CI
Malignant lymphoma	63	53	116	33 (28.4%)	20.5-37.6
Multiple myeloma	31	22	53	16 (30.2%)	18.3-44.3
Myeloid/lymphoid leukemia	26	15	41	31 (75.6%)	59.7-87.6
Myelodysplastic syndrome	13	3	16	7 (43.8%)	19.8-70.1
Total	133 (58.8%)	93 (41.2%)	226	87 (38.5%)	32.1-45.2

CI, confidence interval.

Table 2. Details of infection-associated fever sources and adverse impact on the chemotherapy schedule

Infection-associated fever sources	No. of patients[*1]	%	Duration required for diagnosis of the fever source[*2]	No. of patients with chemotherapy delay
			Mean ± S.D. (Range) (days)	
Catheter-related blood stream infection	6	6.9	3.83 ± 4.75 (1, 13)	0
Pneumonia	6	6.9	2.67 ± 1.51 (1, 5)	0
Dental focal infection	4	4.6	14.00 ± 10.65 (1, 27)	0
SSI associated with tooth extraction	(2)	2.3	20.00 ± 9.90 (13, 27)	1
Acute marginal periodontitis	(1)	1.1	1	0
Pericoronitis of the wisdom tooth	(1)	1.1	15	0
Pharyngitis	4	4.6	4.75 ± 5.56 (1, 13)	1
Urinary tract infection	2	2.3	1.50 ± 0.71 (1, 2)	0
Upper respiratory infection	1	1.1	4	0
Viral hepatitis	1	1.1	13	0
Peritonsillar abscess	1	1.1	12	0
Total	25/87 (28.7%)		5.92 ± 6.56 (1, 27)	2

[*1] ( ) shows the number of patients with dental focal infection; [*2] Duration from the day of fever onset to the day of final diagnosis of fever sources. SSI, surgical-site infection.

in approximately 40% patients with hematologic malignancies. Notably, among the patients who developed fever after chemotherapy, nine (10.3%) were presumably caused by head and neck infections and approximately 5% patients experienced fever because of DFIs; moreover, chemotherapy was postponed because of fever in one patient. To our best knowledge, this is first report to investigate the incidence of DFI in febrile patients with hematologic malignancies who required chemotherapy.

In this study, one patient exhibited acute periodontitis with fever after initiation of chemotherapy; early diagnosis was possible because of severe cellulitis in the buccal region that was evident visibly. In contrast, the diagnosis was late in the other three patients; this included one case of delayed chemotherapy because the possibility of oral cavity-related problem was not initially listed as an option. Tooth extraction before chemotherapy not only helps to extract decayed tooth and treat periodontitis, it also helps in eliminating the DFIs in patients anticipated to experience myelosuppression (*9*). The patient in whom chemotherapy was delayed underwent tooth extraction to remove the infected foci against chronic apical periodontitis of the mandibular first molar approximately 2 weeks before chemotherapy; follow-up was terminated temporarily before initiating chemotherapy because no tooth extraction-related adverse events were observed and the patient could self-administer oral health care. However, fever developed 2 days after initiation of chemotherapy with a sudden decline of physical strength; therefore, the second cycle of chemotherapy was postponed for approximately one week because of prolonged sepsis caused by late onset SSI associated with tooth extraction. Fortunately, the sepsis symptoms were relatively mild and did not affect the chemotherapy schedule in other patients with tooth extraction-associated SSI and pericoronitis. However, these symptoms may hinder administration of chemotherapy because oral hygiene was extremely poor in the affected patients and a severe infection was suspected because of whole-body weakness that accompanied chemotherapy. Based on these observations, we suggest that long-term follow-up (at least during chemotherapy administration) should be performed for patients undergoing tooth extraction, even if the surgical site indicates a good prognosis. Additionally, oral cleaning should be performed regularly even after initiating chemotherapy.

This study had several limitations. First, DFI-associated fever may have been present in patients with FUO. Previously, many reported cases have been diagnosed as FUO after chemotherapy and have demonstrated progression without a clear etiology (*10*). In our study, 21.8% patients who experienced fever were diagnosed with FUO. Among them, definitive intraoral findings were only available in 7/19 (36.8%) patients who visited our department; possibly, many of the patients with FUO may have had DFI. Second, in our study, DFI-related sepsis was not proven bacteriologically; diagnosis was established based on clinical findings. Although the sequential organ failure assessment score is used for the diagnosis of sepsis, detection of microorganisms in blood culture is not essential (*1*). Blood cultures are frequently obtained in patients with serious infections, and they play an important role in identifying the source of infection. In this study, blood cultures were negative in all four cases of DFI-associated sepsis; however, from the clinical findings, the oral cavity was identified as the source of the infection. According to a previous study, of the 1,015 patients with fever (≥ 38˚C), only 128 (12.6%) had clinically significant positive blood cultures, excluding contamination (*11*), indicating that even in patients with sepsis, the causative microorganism cannot always be detected by blood culture. These observations suggest that comprehensive assessments based on clinical findings, including oral and maxillofacial regions, are needed to identify whether an infection reflects fever even if the blood culture results are negative.

Although fever has been usually regarded as a sign of infection (*10*), many types of fever may occur without infection, such as FUO, FN, tumor-related fever, and drug-related fever (*10,12-15*); these were also analyzed in this study. Because differential diagnosis of the etiology of fever after chemotherapy is diverse in patients with hematologic malignancies, further investigation of each symptom is necessary.

In conclusion, although systemic morbidity caused by DFIs is rare, medical and dental specialists should closely monitor oral infections in patients with hematologic malignancy undergoing chemotherapy manifesting fever with unclear source.

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Ethical Approval

All investigations were performed according to the protocols that were reviewed and approved by the ethical committee of National Center for Global Health and Medicine (NCGM-G-001791-02); the requirement for informed consent was waived because of the retrospective study design. We also conducted in accordance with the tenets of the Declaration of Helsinki.

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Metoclopramide versus sumatriptan in the treatment of migraine in the emergency department: a single-center, open-label, cluster-randomized controlled non-inferiority trial

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Abstract: Migraine is a common disease seen in the emergency department (ED). Triptans, which are recommended in therapeutic guidelines for migraine, have some contraindications and possible severe side effects. Metoclopramide, which is commonly used as an antiemetic, also seems to have pain-relieving effects for migraine. In this article, we will introduce a study in progress, which investigates whether metoclopramide 10 mg intravenously (IV) is non-inferior to sumatriptan 3 mg subcutaneously (SQ) as migraine treatment in the ED. This study is a single-center, open-label, cluster-randomized controlled trial of 80 patients with migraine attacks to investigate the non-inferiority of metoclopramide to sumatriptan. The patients will be cluster-randomized monthly into metoclopramide 10 mg IV and sumatriptan 3 mg SQ arms. The primary outcome will be change in Numerical Rating Scale score for headache at 1 h after baseline. In discussion, if our hypothesis is confirmed, metoclopramide can be considered as first-line medication for migraine attacks in ED settings.

***Keywords*:** study protocol, emergency department, pain management, primary headache

Migraine is one of the most common diseases among young and middle-aged people and is the third leading cause of disability in people under 50 years of age according to Global Burden of Disease 2015 (*1*). The annual prevalence of migraine in Japan is 8.4% (*2*), and many migraine patients present to the emergency department (ED). The pathophysiology of migraine has not been definitively elucidated, and there are two theories of the origin of the pain: the peripheral origin theory from cerebral vascular and trigeminal nerve endings, and the central origin theory from brainstem. It has been shown that sensitization to non-nociceptive stimuli occurs in both peripheral and central regions, and it has been shown that nitric oxide, histamine, serotonin, glutamate, dopamine, and calcitonin gene-related peptide (CGRP) are involved in this pathology (*3,4*).

A variety of parenteral medications are used for acute migraine in the ED, but previous studies have indicated that no medication provides rapid and complete relief of pain and associated symptoms without side effects (*5,6*). The clinical guidelines recommend triptans as first-line therapy for moderate to severe migraine attacks (*7,8*). Triptans are serotonin receptor: 5-HT1B/1D receptor agonists, which act on vascular smooth muscle for promotion of vasoconstriction and act on the trigeminal nerve for pain relief. However, some ED doctors hesitate to use triptans because of contraindications, such as a history of ischemic disease or uncontrolled hypertension, and possible side effects, such as chest pressure.

Meanwhile, metoclopramide, a dopamine antagonist, is frequently used for patients with nausea in ED settings in Japan because of its effectiveness, low cost, and few contraindications. It was reported that the frequency of alleles of the dopamine D2 receptor gene was increased in patients with a diagnosis of migraine. Dopamine antagonists act on postsynaptic cells especially in the limbic system and basal ganglia, and have sympathetic inhibition, anti-serotonin, anticholinergic, and antihistamine effects, so are expected to be effective against migraine mechanistically (*9*). Previous studies which compared metoclopramide to other agents for migraine therapy are shown in Table 1.

Previous studies have revealed that both metoclopramide and sumatriptan are more effective than placebo for migraine (*10,11*). One study that compared the effects of metoclopramide and sumatriptan for migraine found no significant difference in pain relief at 2 h after administration (*12*). However, the standard and recommended dose of metoclopramide for treatment for migraine is 10 mg, and the dose of both medications used in the past study was higher than the usual dose recommended for Japanese patients.

Table 1. Studies comparing Metoclopramide to other agents for migraine therapy

Study first author (Year)	Treatment	Control	%Pain Relief
Coppola (1995)	MTC 10 mg IV	PCB IV	48 *vs*. 29
Tek (1990)	MTC 10 mg IV	PCB IV	67 *vs*. 19
Cete (2004)	MTC 10 mg IV	PCB IV	52 *vs*. 35
Ellis (1993)	MTC 10 mg IV	PCB IV	88 *vs*. 31
Cicek (2004)	MTC 10 mg IV	PCB IV	85 *vs*.56
Friedman (2008)	MTC 20 mg IV	PCZ 10 mg IV	78 *vs*. 87
Haugh (1992)	MTC 10 mg IV	DHE 1 mg IV	38 *vs*. 38
Benjamin (2014)	MTC 10 mg IV	VPT 1 g IV	63 *vs*. 40
Benjamin (2014)	MTC 10 mg IV	KET 30 mg IV	63 *vs*. 54
Friedman (2005)	MTC 20 mg IV up to 4 times	STP 6 mg SQ	73 *vs*. 47

DHE, dihydroergotamine; IV, intravenously; KET, ketorolac; MTC, metoclopramide; PCB, placebo; PCZ, prochlorperazine; SQ, subcutaneously; STP, sumatriptan; VPT, valproate.

Table 2. Eligible criteria

Inclusion Criteria
1. Informed consent obtained from the patient.
2. Age 20-65 years.
3. Satisfies the criteria for migraine according to the International Classification of Headache Disorders of the International Headache Society, third beta edition. Time duration can be excluded because of the emergency setting (*14*).
4. More than moderate headache intensity, having a great deal of difficulty doing daily activities at presentation.

Exclusion Criteria
1. Judged as having a high likelihood of secondary headache
2. Temperature ≥ 38.0℃
3. A new objective finding of neurological abnormality
4. History of myocardial infarction or suspected ischemic heart disease
5. History of cerebrovascular disease or transient ischemic attack
6. History of peripheral vascular disorder
7. Uncontrolled hypertension or systolic blood pressure > 180 mmHg at presentation
8. Severe liver dysfunction
9. Suspected gastrointestinal bleeding, perforation, or obstruction
10. Suspected pheochromocytoma
11. Use of an ergotamine derivative, other kind of triptan, or monoamine oxidase (MAO) inhibitor
12. Pregnancy or breastfeeding
13. Allergy to any of the investigational medications
14. Participation judged to be inappropriate by emergency physicians

The recommended and approved doses in Japan are metoclopramide 10 mg intravenously (IV) and sumatriptan 3 mg subcutaneously (SQ) for safety. So, in this study we will investigate whether IV metoclopramide 10 mg, which is an eighth of the dose used in the previous study, is non-inferior to SQ sumatriptan 3 mg, which is half of the dose used in the previous study, for acute migraine attack in the ED setting. Metoclopramide is less expensive and has fewer side effects, and is more widely and easily used in the ED, so it is considered to be a possible standard care for migraine in the ED if metoclopramide is shown to be noninferior to sumatriptan. Therefore, we are conducting a study to assess whether metoclopramide 10 mg IV is non-inferior to sumatriptan 3 mg SQ as treatment for acute migraine attack in the ED setting.

Study design, setting, and patients: This is a single-center, prospective, open-label, cluster-randomized controlled, non-inferiority trial (Trial registration: jRCT registration number: jRCTs031190007; Registered on 5 April 2019). The cluster is the month, and the study period will be 36 months, so there will be 36 clusters. This trial is performed in the ED of the Center Hospital of the National Center for Global Health and Medicine in Japan. About 11,000 patients are emergently transported to the ED annually. Patients emergently transported to the ED for headache are eligible to participate if they fulfill the eligibility criteria in Table 2.

Interventions: After providing informed consent, participants are allocated to one of the two treatments according to the month. Participants in the metoclopramide arm receive metoclopramide 10 mg IV. Participants in the sumatriptan arm receive sumatriptan 3 mg SQ.

Outcomes: Primary outcome is change in headache pain intensity 1 h after baseline, measured with the Numerical Rating Scale for Pain (NRS) (*13*). Secondary endpoints are change in NRS score 30 min after medication administration, headache relief 1 h after medication administration, defined as the patient's description of headache from severe or moderate to either mild or none. Concomitant symptoms 1 h after medication administration, time duration from study medication administration to leaving the ED, receipt

of rescue medication during the ED visit, and adverse events are also secondary outcomes.

Sample size: A previous study indicated an expected NRS pain score reduction of 6 and 5 in the metoclopramide and sumatriptan groups, respectively (*14*). Even though the doses of the study medication were not the same, findings from other studies indicated that a high metoclopramide dose was no better than a lower dose for pain relief (*15*). Based on previous data, we set the standard deviation as 3 NRS points. The non-inferiority margin is set as -1.0 NRS points, because findings from a previous study indicated that a 1.3-NRS-point between-group difference was a valid and reproducible minimum clinically significant change in the ED (*16*). Thus, a sample size of 37 in each group is calculated to be sufficient with a one-sided α of 0.025 and a power of 0.8 (*17*). Taking potential dropout rates into account, a sample size of 40 in each group is eventually determined.

Randomization and concealment: Metoclopramide and sumatriptan require different routes of administration, so for patient safety in the busy ED, randomization is performed on a monthly basis and neither physicians nor participants are blinded. The monthly allocation is done with computer-generated random numbers. With cluster randomization by month, participants can receive the study medication quickly after enrollment.

Statistical analysis: All randomized participants who fulfill the eligibility criteria and sign the informed consent form will be included in the intention-to-treat (ITT) set. All participants who take either study medication will be included in the safety analysis set. Analysis of adverse events will be based on the safety analysis set. For the primary outcome, we will report the within-group improvement in NRS pain score between baseline and 1 h. Student's *t* test will be used to compare mean differences in NRS score and lower one-sided 95% confidence interval (CI), along with a one-sided $p < 0.025$. Statistical analyses will be performed using JMP statistical software, version 14.0.0 (SAS Institute Inc., Cary, NC).

This study is a non-inferiority study of metoclopramide to sumatriptan for acute migraine attack in the ED setting. Although the efficacy of metoclopramide for migraine has previously been reported, metoclopramide is not yet approved as a treatment for migraine in Japan.

Metoclopramide is less expensive and has fewer side effects, and is more widely and easily used in the ED than sumatriptan. So, if our hypothesis that metoclopramide is not inferior to sumatriptan for pain relief of migraine attack in the ED is confirmed, metoclopramide can be considered as first-line medication for migraine attacks in ED settings.

Finally, we discuss the potential limitations of this study. First, neither participants nor physicians will be blinded to the treatment because each medication requires different routes of administration. NRS score is a subjective index, but it is not affected by overcrowding or the presence of other patients in the ED. Some previous studies set the primary outcome as difference in NRS score at 80 min or 120 min after medication. A pilot retrospective survey of migraine patients at the ED of our hospital indicated that the mean duration of ED visit after medication is 75 min. Thus, we set the primary outcome as the improvement in NRS score at 1 h. A second limitation is that randomization will be performed on a monthly basis rather than on a participant basis. The frequency or severity of migraine attacks have no seasonal variation, so this monthly cluster randomization may not lead to bias. A monthly cluster randomization thus enables quick administration of study medications.

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LETTER

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Nerve vascularity in free vascularized nerve flaps

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Abstract: The blood supply of peripheral nerves consists of a complex internal vessels' network, feeding external vessels and the interlinking vasa nervorum. Patients with nerve damage may require nerve substitution. While the commonly performed avascular nerve grafts obtain vascularization only from random and slow inosculation into the vasa nervorum, their insufficient revascularization causes loss of the graft's potential due to central necrosis. This gets more relevant with the larger diameter of nerves injured. Examples for neurovascular flaps are the lateral femoral cutaneous nerve vascularized *via* the superficial circumflex iliac artery perforator (LFCN-SCIP) flap or the iliohypogastric nerve graft vascularized *via* the superficial inferior epigastric artery (SIEA). LFCN-SCIP shows a well concealed donor scar site with a maintained vascularization and a minor donor site morbidity. Therefore, the guaranteed axial nerve vascularity in LFCN-SCIP makes it a preferred autologous vascularized nerve therapy for peripheral nerve defects. A further option example is the anterior lateral thigh (ALT) flap with the LFCN.

***Keywords*:** perforator, supermicrosurgery, SCIP, nerve reconstruction, microsurgery, flap, vascularized nerve

Soft tissue defects may often require nerve reconstruction. This can occur after cancer resection, after trauma or iatrogenically. Based on the reconstructive ladder, short defects of nerves can first be repaired *via* primary coaptation or avascular nerve grafts (*e.g*. sural nerve graft).

Nerve grafting provides the necessary extracellular matrix along with viable Schwann-cells for the proximal axons to grow along, until the distal target is reached. These nerve grafts (*e.g*. sural nerve graft) are vascularized only *via* inosculation from the surrounding tissue, which limits viability of their core, and thus their potential (*1*). For short distances (up to 3 cm), nerve allografts (Axogen®) are commercially offered, yielding no superiority to autologous material except preventing donor site morbidities. Additionally, they offer no additional benefit regarding vascularization. Scarring, long or thick nerve defects require preferably vascularized nerve reconstruction (*2*), where the vascular structure (external and internal vessels) are intact, which provides the optimal condition for the proximal nerves to grow (*3*). This vascularized nerve reconstruction can be in the form of vascularized nerve grafts (*e.g*. anterior interosseous nerve graft or ulnar nerve graft), neurovascular flaps (*e.g*. lateral arm flap), and neurocutaneous flaps (where the nerve is a guide for skin vascularization).

The vessels of a peripheral nerve are commonly categorized into the intrinsic and the extrinsic vascular systems (*4*). The intrinsic system is the dense mesh, which nourishes the nerve. It consists of the longitudinal microvessels within the endoneurium. It is very rich and allows a nerve to be supplied for up to 40 cm. Yet, the intrinsic system receives its supply from extrinsic segmental nutritive vessels, each of them not vital, because there is a huge redundancy among them for the vital nervous system. Taylor *et al*. categorize them morphologically based on parallel vessels with multiple perforators into the nerve, or scattered perforators from nonparallel vessel sources (*5*). Preserving any type of extrinsic vessels from the surrounding feeder tissue sustains the intrinsic nerve vascularity for relatively long distance.

We usually harvest vascularized nerves as a free neurovascular flap, which is axially based, for example lateral femoral cutaneous nerve (LFCN). The superficial circumflex iliac artery perforator (SCIP) accompanies the LFCN (*6,7*). It can also be harvested based on the deep circumflex iliac artery, which lies deeper and harvesting is more invasive (*5*). LFCN nourished by the SCIP (LFCN-SCIP) flap can be harvested as a long nerve flap with a guaranteed axial continuous vascularization avoiding central nerve necrosis. It is possible, from our experience, to harvest an over 15 cm long nerve graft. Intraoperatively it is assessed for viability by bright colored bleeding at the nerve endings. The superficial circumflex iliac artery originates from the femoral artery and this flap does not require main artery

sacrifice. Compared to common nerve flaps, LFCN-SCIP flap shows a well concealed donor scar site and less invasiveness.

In the inferolateral abdominal region, there are two workhorse neurovascular flaps, which are capable of providing a vascularized nerve graft with or without composite tissue coverage. These are the LFCN-SCIP flap or the superficial inferior epigastric artery (SIEA) flap with the subcostal nerve (T12) respectively the iliohypogastric nerve (IHN).

IHN-SIEA was applied for a volar nerve defect of a 55-year-old patient with posttraumatic composite tissue defect of the palm, including a 3.6 cm nerve defect of the 3rd common digital, ulnar and radial digital nerves. The IHN was vascularized through the anastomosis of the SIEA (diameter = 0.5 mm) to the 3rd common digital artery stump. After 6 months, the 2-point discrimination inside the flap was restored up to 4.17 mm distally, as compared to 2 mm in the control (*8*).

LFCN based on SCIP flap can be applied to any kind of nerve reconstruction. The advantage of SCIP flap is that it can be harvested as vascularized composite graft less invasively. It is especially suitable for hand complex injuries. Of course, it is also available for simple nerve reconstruction as in facial palsy. However, one major further benefit is the possibility of chimeric flap design including muscle, bone and skin for further compound defect reconstruction (*9*).

Another example is the LFCN-anterior lateral thigh (LFCN-ALT) flap, which is larger in the reconstructed nerve diameter. A 70-year-old female with recurrent, extremely painful CTS neuroma in continuity was treated with a 2 cm LFCN-ALT neurovascular flap, especially due to recurrent scarring. The severe pain disappeared completely and the motor impairment of median nerve improved after 5 months (*10*).

In conclusion, nerve vascularity must be considered, when treating peripheral nerve defects. Neurovascular SCIP flap is preferable for vascularized nerve flap transfer due to its axial pattern, versatility and hidden donor site. Another option is the ALT flap with the larger LFCN sensory nerve.

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NCGM GHM Global Health & Medicine

Information for Authors

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Global Health & Medicine is (Print ISSN 2434-9186, Online ISSN 2434-9194) is an international, open-access, peer-reviewed journal dedicated to publishing high-quality original research that contributes to advancing global health and medicine, with the goal of creating a global information network for global health, basic science as well as clinical science oriented for clinical application.

We encourage submission of original research findings in the fields of global health, public health, and health care delivery as well as the seminal and latest research on the intersection of biomedical science and clinical practice.

2. Types of Articles

Types of Articles	Words in length (excluding references)	Figures and/or Tables	References
Original Articles	~5,000	~10	~50
Brief Reports	~3,000	~5	~30
Reviews	~8,000	~10	~100
Mini reviews	~4,000	~5	~50
Policy Forum articles	~3,000	~5	~30
Communications	~2,000	~2	~20
Perspectives			
Comments			
Correspondence			
Editorials	~1,000	~1	~10
Letters	~1,000	~1	~10
News	~800	~1	~5

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Keywords: 3~6 words

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2. Main manuscript
3. Figures
4. Supplementary Data, if appropriate

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2. Abstract
3. Main Text
4. Acknowledgments
5. References
6. Tables
7. Figure Legend
8. List of Supplementary Data, if appropriate

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