Blood cytokine, chemokine and gene expression in cholestasis patients with intractable pruritus treated with a molecular adsorbent recirculating system: A case series

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BACKGROUND: The molecular adsorbent recirculating system (MARS) is an albumin-dialysis modality that has been investigated predominantly in patients with acute and acute-on-chronic liver failure.
OBJECTIVES: To report the clinical efficacy and safety of MARS therapy for intractable pruritus in cholestasis patients with stable chronic liver disease, characterizing the impact of MARS on cytokine levels and on the transcriptome in the blood compartment.
METHODS: MARS therapy was performed on three patients with cholestatic liver disease using 8 h runs for two consecutive days. The expression levels of 65 cytokines/chemokines and 24,000 genes were profiled by Luminex (Luminex Corporation, USA) and microarray, respectively.
RESULTS: A quality-of-life assessment demonstrated a marked improvement during therapy, which was sustained in two of three patients. No bleeding or infectious complications were observed. Bile acid levels were markedly reduced following MARS (mean [± SD] pretreatment 478.9±112.2 µmol/L versus post-treatment 89.7±68.8 µmol/L). Concordant decreases in cytokine/chemokine levels were noted for interleukin (IL)-1beta, IL-2, IL-6, IL-8, IL-12 (p40), RANTES, tranforming growth factor-alpha, tumour necrosis factor-alpha and thrombopoietin following MARS. On microarray profiling, biologically relevant concordant changes among all patients were evident for 20 different genes (10 upregulated and 10 downregulated). The upregulation of several potentially immune suppressive/regulatory genes (eg, early growth response 3 [*EGR-3*], ephrin-A2 [*EFNA2*] and serum amyloid A1 [*SAA1*]), concurrent with downregulation of genes involved in innate immunity (eg, toll-like receptor 4 interactor with leucine-rich repeats [*TRIL*]) and inflammation (eg, ephrin receptor B1 [*EPHB1*]), was observed.
CONCLUSIONS: This investigative approach offers new insights into intractable pruritus and suggests future therapeutic targets. The clinical benefit of MARS in cholestasis patients with intractable pruritus may not exclusively result from filtration of pruritogens, but also from systemic changes in cytokine/chemokine levels and changes in gene expression of blood cells.

Key Words: *Cholestasis; Cytokine; Gene expression; Intractable pruritus; MARS*

Les cytokines sanguines, les chémokines et l'expression génétique chez les patients ayant une cholestase entraînant un prurit réfractaire traité au moyen d'un système de recirculation adsorbant moléculaire : une série de cas

HISTORIQUE : Le système de recirculation adsorbant moléculaire (SRAM) est une modalité de dialyse de l'albumine qui a surtout été mis à l'essai chez les patients ayant une insuffisance hépatique aiguë ou aiguë à chronique.
OBJECTIFS : Rendre compte de l'efficacité et de l'innocuité cliniques d'une thérapie par SRAM pour soigner un prurit réfractaire chez les patients ayant une cholestase et une maladie hépatique chronique stable, afin de caractériser les répercussions du SRAM sur les taux de cytokine et sur le transcriptome du compartiment sanguin.
MÉTHODOLOGIE : Trois patients ayant une maladie hépatique cholostatique ont reçu un traitement par SRAM toutes les huit heures pendant deux jours consécutifs. Les chercheurs ont profilé le taux d'expression de 65 cytokines et chémokines et de 24 000 gènes par Luminex (Luminex Corporation, États-Unis) et par microréseaux, respectivement.
RÉSULTATS : Une évaluation de la qualité de vie a démontré une amélioration marquée pendant le traitement, qui s'est maintenue chez deux des trois patients. Les chercheurs n'ont observé aucune complication hémorragique infectieuse. Les taux d'acide biliaire étaient considérablement plus faibles après le SRAM (moyenne [±ÉT] de 478,9±112,2 µmol/L avant le traitement par rapport à 89,7±68,8 µmol/L après le traitement). Les chercheurs ont constaté des diminutions concomitantes des taux de cytokine et de chémokine de l'interleukine (IL)-1bêta, de l'IL-2, de l'IL-6, de l'IL-8, de l'IL-12 (p40), des protéines RANTES, du facteur de croissance transformant alpha, du facteur de nécrose tumorale alpha et de la thrombopoïétine après le SRAM. Au profilage par microréseaux, tous les patients présentaient des changements à 20 gènes différents concordants sur le plan biologique (dix surexprimés et dix sous-exprimés). Ils ont observé la surexpression de plusieurs gènes au potentiel immunosuppressif ou régulateur (p. ex., réponse de croissance précoce 3 [EGR-3], éphrine A2 [EFNA2] et amyloïde sérique A1 [SAA1]), conjointement avec une sous-expression des gènes participant à l'immunité innée (p. ex., récepteur de type Toll 4 comportant des motifs répétés riches en leucine [TRIL]) et à l'inflammation (p. ex., récepteur d'éphrine B1 [EPHB1]).
CONCLUSIONS : Cette démarche de recherche donne un nouvel éclairage sur le prurit réfractaire et ouvre la voie à de futures cibles thérapeutiques. Les bienfaits cliniques du SRAM chez les patients ayant une cholestase et un prurit réfractaire ne résultent peut-être pas exclusivement du filtrage des pruritogènes, mais également de changements systémiques aux taux de cytokine et de chémokine ainsi que de l'expression génique des globules sanguins.

Pruritis (itch) is one of the most common debilitating symptoms in patients with cholestatic liver disease. In these patients, pruritus has a significant impact on quality of life (QoL), mental health and, in severe, otherwise intractable cases, may require assessment for liver transplantation. However, these patients are often not prioritized for organ allocation in systems based on the current listing criteria (ie, Model for End-stage Liver Disease score).

The physiopathology of cholestatic pruritus has not been completely elucidated. Current treatment options for intractable pruritus often target underlying cholestatic liver disease (ursodeoxycholic acid) or the removal of bile acids (1-6). Anion exchange resins (cholestyramine and colesevelam) and enzyme-inducing agents (rifampin) act mainly by increasing the clearance of bile acids (7-9). However, cholestatic pruritus may not improve despite marked reduction in bile acid

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levels; conversely, improvement achieved with these medications may be sustained regardless of rebound increases in bile acids on cessation of therapy. Other therapies (naloxone, naltraxone, nalmefene) prevent the binding of endogenous opioid agonists, the levels of which are elevated in cholestasis (10-13). Recently, the pruritogenic potential of lysophosphatidic acid has been examined in intrahepatic cholestasis of pregnancy and primary biliary cirrhosis (PBC) (14). To date, medical therapies have not been developed to specifically target this pathway.

More invasive therapeutic modalities have been used when conventional approaches have proven ineffective. Results for plasmapheresis have been positive but lack large patient numbers (15-17). Albumin dialysis-based therapies, such as the molecular adsorbent recirculating system (MARS, Gambro, Sweden), have previously been investigated predominantly in acute and acute-on-chronic liver failure, in which the removal of both albumin-bound (ie, bilirubin, bile acids, middle- and short-chain fatty acids) and small water-soluble molecules (ie, ammonia, creatinine, cytokines, urea) have been characterized. MARS has also been used in patients with cholestatic chronic liver disease for relief of intractable pruritus. Several case series have shown improvement in biochemical parameters (bile acids) and in QoL by visual analogue scores in these patients without, nonetheless, offering new insights on how the clinical benefit may be obtained.

While MARS therapy appears to have a significant effect on bile acids, we hypothesized that there may be novel pruritogenic pathways that may be modified by albumin dialysis therapy. In the present pilot study, we report the clinical efficacy and safety of MARS therapy for intractable pruritus in three cholestasis patients with stable chronic liver disease, characterizing the changes in cytokine and chemokine levels, and the impact of MARS in gene expression observed in the blood compartment.

METHODS

Inclusion criteria

The study was approved by the Health Research Ethics Board of the University of Alberta (Edmonton, Alberta). Adult patients (>18 years of age) with PBC or benign recurrent intrahepatic cholestasis (BRIC) with intractable pruritus, defined by bile acid levels >300 µmol/L and a European Quality of Life-5 Dimensions (EQ-5D) visual analogue score ≤30/100 (0 = worst) were considered eligible. All patients were receiving ursodeoxycholic acid. To be considered eligible, patients were required to be either refractory to or intolerant of medical therapy consisting of cholestyramine (8 g/day), rifampin (300 mg/day to 600 mg/day) and naltrexone (50 mg/day). Patients were excluded if they had other causes of intractable pruritus (eg, drug reactions).

MARS

MARS therapy was performed in the General Systems Intensive Care Unit at the University of Alberta Hospital. Before commencing MARS, each patient received a temporary 16 cm (12 Fr) hemodialysis catheter (Mahurkar/Coviden, USA) inserted in the right internal jugular vein under ultrasound guidance. MARS therapy was performed in conjunction with intermittent hemodialysis (Integra, Gambro, Sweden). Bloodlines were primed with 2 L of 0.9% normal saline and 5000 units/L heparin. Blood flow rates were 250 mL/min. Four hundred millilitres of 25% albumin was used in the albumin dialysate, which was run at a rate of 250 mL/min countercurrent to the blood circuit. The albumin dialysate was recirculated through charcoal and anion-exchange columns before undergoing standard hemodialysis. Bicarbonate dialysis (standard composition: sodium 140 mmol/L, potassium 4 mmol/L, sodium bicarbonate 30 mmol/L) was run at 500 mL/min. Patients targeted an even fluid balance while on MARS. Heparin was used for anticoagulation while on MARS therapy (bolus 500 to 1000 units, infusion 250 units/h to 1000 units/h) and was run to target an activated clotting time of between 180 s and 200 s. Each patient underwent two MARS runs consisting of 8 h per run on consecutive days (total of 16 h). During MARS, a dedicated certified MARS trained nurse was responsible for operating the device while a second nurse was responsible for other aspects of patient care.

Sample collection and storage

Blood samples were collected from indwelling venous lines immediately before the first treatment (ie, pre-MARS) and immediately at the end of the second MARS run (ie, post-MARS). Serum samples were obtained by centrifugation within 1 h of blood collection, aliquoted and stored at −80°C until use. PAXgene RNA tubes (PreAnalytiX GmbH, Switzerland) were used for prompt RNase inactivation of whole blood specimens. Blood in PAXgene tubes were stored at −80°C until use.

Microarray analysis

Nimblegen Gene Expression 4×72K Hs18.0 arrays (Roche NimbleGen, USA) containing 24,000 different probes for human genes were used to profile messenger RNA (mRNA) expression in peripheral blood specimens. RNA extraction and microarray processing were performed at the Alberta Transplant Applied Genomics Centre (University of Alberta). Briefly, RNA was extracted using the PAXgene Blood RNA Kit (PreAnalytix, Switzerland) and 200 ng of total RNA was used for preparation of a complementary DNA (cDNA) library that was subjected to unbiased linear preamplification over 17 polymerase chain reaction cycles (WTA2 kit, Sigma Aldrich, USA). Labelling of 1 µg of the resulting cDNA with Cy3 was performed using the One-Color DNA Labeling kit (Roche Nimblegen, Sweden) according to manufacturer's instructions. These were subsequently hybridized onto the microarray slides and scanned on a microarray scanner (MS200, Roche Nimblegen). Data extraction was performed using Nimblescan software (Roche Nimblegen), having arrays being robust multi-array average summarized. Each patient's pre- and post-MARS samples were run concurrently on the same array chip.

Cytokine analysis

A panel of 65 cytokines and chemokines (EGF, Eotaxin, FGF-2, Flt-3 ligand, fractalkine, G-CSF, GM-CSF, GRO, interferon [IFN]-alpha 2, IFN-gamma, interleukin [IL]-10, IL-12 [p40], IL-12 [p70], IL-13, IL-15, IL-17, IL-1ra, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-3, MDC [CCL22], MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, regulated on activation, normal T cell expressed and secreted [RANTES], transforming growth factor [TGF]-alpha, tumour necrosis factor [TNF]-alpha, TNF-beta, VEGF, sCD40L, sIL-2Rα, MCP-2, MCP-4, ENA-78/CXCL5, SDF-1α+β/CXCL12, BCA-1/CXCL13, I-309/CCL1, MIP-1δ/MIP-5/CCL15, TARC/CCL17, 6Ckine/CCL21, EOTAXIN-2/CCL24, EOTAXIN-3/CCL26, CTACK/CCL27, IL-23, LIF, TPO, TRAIL/TNFSF10, SCF, TSLP, IL-20, IL-21, IL-28A, IL-16, IL-33/NF-HEV) were simultaneously measured in plasma samples using a MILLIPLEX MAP Human Cytokine/Chemokine kits (Millipore, USA) according to the manufacturer's protocol. The multiplexing analysis was performed using the Luminex 100 system (Luminex, USA) by Eve Technologies Corporation (Canada).

Autotaxin

Serum autotaxin activity was determined with a choline-release assay using lipophosphatidylcholine as a substrate, as described in detail elsewhere (18) and performed at the Tytgat Institute for Liver and Intestinal Research at the University of Amsterdam (Rotterdam, The Netherlands).

Data analysis

Microarray data analysis was performed using GeneSpring GX 11 software (Agilent Technologies, USA). Arrays were baseline transformed to the expression of beta-actin (*ACTB*, Gene ID 60) on each chip. Fold-change was used to characterize differences in gene expression in each patient individually, excluding the 20% of probes with the lowest raw intensity values. Cytokine and chemokine

TABLE 1
Quality of life and biochemical characteristics of patients pre- and post-MARS therapy

	Patient			Fold change,
	1	2	3	mean
Diagnosis	PBC	PBC	BRIC	
MELD score	11	7	20	
EQ-5D score (VAS)				5.33↑
Pre	10	20	20	
Post	90	70	70	
Bile acids, µmol/L				5.43↓
Pre	555.1	531.5	350.1	
Post	48.2	169.1	51.8	
Ammonia, µmol/L				1.11↑
Pre	24	44	27	
Post	42	37	20	
Alanine aminotransferase, U/L				1.05↑
Pre	151	42	59	
Post	152	46	62	
Aspartate aminotransferase, U/L				1.12↑
Pre	173	56	69	
Post	159	65	88	
Total bilirubin, µmol/L				1.48↓
Pre	208	28	565	
Post	138	23	303	
Conjugated bilirubin, µmol/L				1.69↓
Pre	125	14	377	
Post	79	10	161	
Alkaline phosphatase, U/L				1.11↓
Pre	481	255	532	
Post	445	239	448	
Gamma-glutamyl transpeptidase, U/L				1.18↓
Pre	73	212	51	
Post	60	190	42	
International normalized ratio				1.07↑
Pre	1.1	1	1.7	
Post	1	1	2.2	
Partial thromboplastin time, s				2.26↑
Pre	31	31	55	
Post	57	126	48	
Hemoglobin, g/L				1.11↓
Pre	96	98	106	
Post	81	91	98	
Platelets, $\times 10^9$/L				1.30↓
Pre	199	114	271	
Post	148	81	231	
Creatinine, µmol/L				1.26↓
Pre	47	80	47	
Post	45	63	30	
Urea, mmol/L				2.94↓
Pre	4.4	5.2	2.6	
Post	1.1	2	<1.0	

Fold-changes are given, with arrows representing fold-change increase (↑) or decrease (↓). BRIC Benign recurrent intrahepatic cholestasis; EQ-5D European Quality of Life-5 Dimensions questionnaire; MARS Molecular adsorbent recirculating system; MELD Model for End-stage Liver Disease; PBC Primary biliary cirrhosis; VAS Visual analogue score

changes were also expressed as fold-change increase (when values post/pre-MARS >1.0) or decrease (−1/[values post/pre-MARS]).

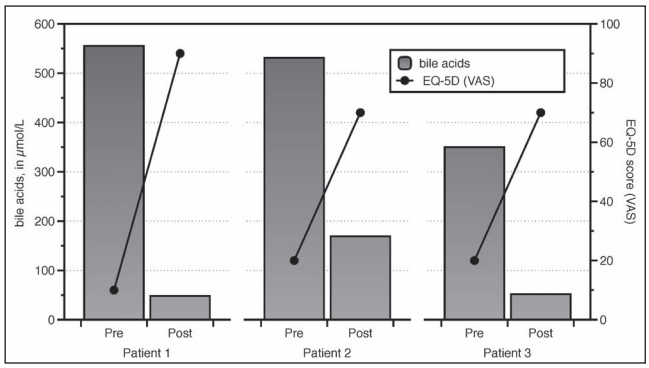

Figure 1) *Bile acid and European Quality of Life-5 Dimensions (EQ-5D) questionnaire changes in patients treated with the molecular adsorbent recirculating system (MARS). The bars indicate bile acid levels while the lines represent scores for the EQ-5D test (visual analogue scale [VAS] component) in relation to MARS treatment (ie, pre/post)*

RESULTS

Effectiveness and safety

Two adult PBC patients and one adult BRIC patient with intractable pruritus underwent two sessions of MARS therapy each during the study period. All patients had failed standard medical therapy before referral for MARS therapy. MARS was administered in 8 h runs for two consecutive days for all patients. Clinical and biochemical outcomes are shown in Table 1. QoL assessment using the EQ-5D visual analogue scale demonstrated the marked improvement obtained during therapy in all three patients (Figure 1: range pre 10 to 20 versus post 70 to 90), which was sustained for the short term (two weeks) in two of three patients. Reductions in mean (± SD) hemoglobin levels (pre 100.0±5.29 g/L versus post 90±8.54 g/L) and platelet levels (pre $194.7\pm78.6\times10^9$/L versus post $153.3\pm75.1\times10^9$/L) post-MARS were not associated with requirement for blood transfusion. No bleeding or infectious complications associated with catheter insertion or therapy were apparent.

Biochemical response to MARS therapy

Biochemical data are also presented in Table 1. Bile acid levels were markedly reduced with MARS therapy (Figure 1: mean pre-MARS 478.9 µmol/L versus post-MARS 89.7 µmol/L). Bilirubin levels were decreased in two of three patients (each had abnormal bilirubin levels pre-MARS treatment). No changes in ammonia levels were observed.

Cytokine and chemokine

Patterns of change in most cytokines and chemokines were complex and largely distinct among the patients. Concordant decreases were recorded for IL-1ß, IL-2, IL-6, IL-8, IL-12 (p40), RANTES, TGF-alpha, TNF-alpha and thrombopoetin, in which levels were reduced post-therapy in all three patients, although by low magnitudes (Table 2). No concordant increases were observed.

Autotaxin activity levels

The activity levels of ectonucleotide pyrophosphatase/phosphodiesterase (autotaxin) were discordant among the patients. While patients 1 and 3 experienced a marked reduction of 37% (17.5 nmol/mL*min to 12.8 nmol/mL*min) and 21% (13.3 nmol/mL*min to 11.0 nmol/mL*min) respectively, patient 2 had low baseline values and showed a mild increase of 16% (7.3 nmol/mL*min to 8.4 nmol/mL*min) in the activity levels of this enzyme associated with MARS therapy.

Genome-scale transcriptome analysis

A comprehensive analysis of mRNA in blood samples was performed through microarray profiling. Up- or downregulation of gene expression for each individual patient was considered biologically relevant when the fold-change was ≥2.0. Concordant changes among the three patients

TABLE 2
Baseline serum cytokine and chemokine levels and concordant fold-changes in patients who underwent MARS therapy

Protein	Gene symbol	Baseline, mean, pg/mL	Fold change		
			Patient 1	Patient 2	Patient 3
IL-1β	*IL1B*	2.72	↓	–2.87	–1.54
IL-2	*IL2*	1.05	–1.15	↓	–1.68
IL-6	*IL6*	5.30	↓	–1.52	–1.11
IL-8	*IL8*	95.01	–1.38	–1.26	–1.62
IL-12 (p40)	*IL12B*	19.84	–1.15	–1.44	–1.19
RANTES	*CCL5*	815.37	–1.43	–1.51	–1.38
Transforming growth factor-α	*TGFA*	3.20	–1.27	–1.65	–1.15
Tumour necrosis factor-α	*TNF*	5.40	–1.34	–1.20	–1.10
Thrombopoietin	*TPO*	452.45	–1.29	–1.07	–1.02

Individual patient fold-changes and mean baseline levels of cytokine/chemokine were determined for values obtained within the linear range of detection. Arrows indicate one of the results obtained outside of the linear range of the assay but yet allowing analysis of the trend (ie, decrease [↓]). Negative values denote fold-change decrease. IL Interleukin; MARS Molecular adsorbent recirculating system; RANTES Regulated on activation, normal T cell expressed and secreted

were apparent for 20 different genes (10 upregulated and 10 downregulated) (Table 3). No transcripts corresponding to profiled serum cytokines or chemokines were found to be affected by MARS therapy in blood cells. Upregulation of several potentially immune suppressive/regulatory genes, such as early growth response 3 (*EGR-3*), ephrin-A2 (*EFNA2*) and serum amyloid A1 (*SAA1*), concurrent with downregulation of genes involved in innate immunity, such as toll-like receptor 4 interactor with leucine-rich repeats (*TRIL*), and in inflammation, such as *EPHB1*, was apparent post-therapy.

DISCUSSION

In the present case series, we described the first Canadian experience using MARS for the treatment of cholestasis patients with intractable pruritus. Our preliminary data confirm the clinical benefit of MARS, in addition to its safety and tolerability. Symptomatic relief and reduced bile acid levels were achieved with MARS therapy in patients with intractable pruritus (Tables 1 and 4). Moreover, aside from its previously characterized filtration capacity of potential pruritogens, the clinical benefit of MARS may also be explained by associated changes in cytokine/chemokine profiles and in gene expression in blood cells.

Nonresident leukocytes infiltrating the skin, including eosinophils and T cells, are believed to play a role in the pathogenesis of chronic pruritus (19). We postulate that changes in gene expression, cytokines and chemokines seen in the blood in association with MARS modulate the cell types with causative roles in pruritus before their migration to the skin.

Cytokine and chemokine changes associated with MARS therapy have not yet been described in the serum of stable cholestatic liver disease patients with intractable pruritus. To the best of our knowledge, the present pilot study was the first to assess this. We found that proinflammatory cytokines and chemokines comprised the bulk of the proteins concordantly decreased in all patients in this series, some of which are known to be cleared by this filtration method. The comparison between the experience with MARS in the present and other clinical scenarios suggests a similar effect of the therapy over cytokine and chemokine levels (Table 5). Interestingly, cytokines, such as IL-6 and TNF-alpha, have been previously implicated in pruritis associated with uremia or HIV infection (20, 21). The lack of a clear correlation between cytokine level changes and their corresponding mRNA in blood cells may suggest their predominant extravascular compartment origin.

MARS therapy in our patients also impacted circulating leukocytes at the transcriptional level. Among the upregulated genes and with the highest mean fold-change (3.73) were *EGR3*, which is a negative regulator of T cell activation (22,23), playing a role in T cell

TABLE 3
Up- and downregulated genes in blood samples of patients who underwent MARS therapy

Gene ID	Gene symbol	Gene name	Mean fold-change	Individual fold change		
				Patient 1	Patient 2	Patient 3
1960	*EGR3*	Early growth response 3	3.73	5.10	1.70	4.39
9865	*TRIL*	TLR4 interactor with leucine-rich repeats	–2.58	–1.51	–4.52	–1.70
375010	*LOC375010*	Ankyrin repeat domain 20 family, member A pseudogene	–2.54	–3.03	–2.65	–1.94
27023	*FOXB1*	Forkhead box B1	2.50	1.62	3.64	2.23
81127	*OR4K15*	Olfactory receptor, family 4, subfamily K, member 15	–2.50	–1.77	–1.71	–4.01
219493	*OR5AR1*	Olfactory receptor, family 5, subfamily AR, member 1	–2.49	–1.64	–3.36	–2.46
153579	*BTNL9*	Butyrophilin-like 9	–2.46	–3.05	–1.58	–2.75
136371	*ASB10*	Ankyrin repeat and SOCS box-containing 10	2.38	1.84	3.15	2.16
6288	*SAA1*	Serum amyloid A1	2.35	1.74	3.58	1.74
25790	*CCDC19*	Coiled-coil domain containing 19	–2.37	–2.54	–2.43	–2.13
1943	*EFNA2*	Ephrin-A2	2.27	2.14	1.83	2.83
2047	*EPHB1*	Ephrin receptor B1	–2.26	–2.44	–1.51	–2.84
4108	*MAGEA9*	Melanoma antigen family A, 9	–2.20	–2.24	–1.64	–2.71
79933	*SYNPO2L*	Synaptopodin 2-like	–2.16	–1.82	–3.14	–1.52
643444	*LOC643444*	Similar to WAS protein homology region 2 domain containing 1-like 1	2.12	2.03	2.44	1.89
2128	*EVX1*	Even-skipped homeobox 1	2.09	2.69	1.85	1.73
392583	*LOC392583*	Hypothetical LOC392583	2.07	1.68	3.00	1.53
2232	*FDXR*	Ferredoxin reductase	2.01	1.53	2.90	1.61
22854	*NTNG1*	Netrin G1	2.01	1.58	2.76	1.68
55701	*ARHGEF40*	Rho guanine nucleotide exchange factor (GEF) 40	–2.01	–2.42	–1.98	–1.64

Values represent fold-change increase (normalized expression post/normalized expression pre) or decrease (−1/[normalized expression post/normalized expression pre]) for each individual patient and genes are sorted according to the absolute mean fold-change observed in the group. ID Identification; MARS Molecular adsorbent recirculating system; TLR Toll-like receptor; WAS Wiskott-Aldrich syndrome

TABLE 4
Summary of publications* on cytokines and/or chemokines in MARS therapy

				Outcomes post-MARS therapy	
Author (reference), year	**Country**	**n**	**Etiology**	**Biochemical**	**Pruritus**
Lisboa et al (present), 2012	Canada	3	PBC (n=2), BRIC (n=1)	↓Bile acids, bilirubin, urea	Improvement in 3/3
Leckie et al (30), 2012	UK	15	PBC (n=11), PSC (n=3), Other (n=1)	↓Bilirubin, ALT, ALP, creatinine	Improvement in 13/15
Pares et al (31), 2010	Spain	20	PBC (n=10), PSC (n=1), Alagille syndrome (n=1), OLT graft rejection (n=8)	↓Bile acid, bilirubin, GGT, cholesterol	Improvement in 19/20
Lemoine et al (32), 2008	France	1	PFIC3 (n=1)	↓Bile acids	Improvement in 1/1
Silvagni et al (33), 2008	Italy	1	Drug-induced + Turner syndrome (n=1)	↓Bile acid	Improvement in 1/1
Novelli et al (34), 2006	Italy	9	HCV (n=9)	↓Bile acid, bilirubin, creatinine	Improvement in 9/9
Montero et al (35), 2006	Spain	3	PBC/AIH (n=1), post-OLT cholestasis (n=1), post-OLT HCV recurrence (n=1)	↓Bilirubin	Improvement 4/4
Saich et al (36), 2005	UK	1	BRIC (n=1)	↓Bile acids	Improvement in 1/1
Ribo et al (37), 2005	Spain	2	post-OLT cholestasis (n=2)	↓Bilirubin	Improvement in 2/2
Bellmann et al (38), 2004	Austria	2	Drug-induced (n=2)	↓Bile acids, bilirubin	Improvement in 2/2
Pares et al (39), 2004	Spain	4	PBC (n=4)	↓Bile acids	Improvement in 4/4
Bellmann et al (40), 2004	Austria	7	post-OLT cholestasis (n=7)	↓Bile acid, AST, GGT	Improvement in 6/7
Macia et al (41), 2003	Spain	3	post-OLT cholestasis (n=1), PBC (n=2)	↓Bilirubin, urea, creatinine	Improvement in 3/3
Doria et al (42), 2003	Italy	3	HCV (n=3)	↓Bile acid	Improvement in 3/3
Sturm et al (43), 2002	France	1	BRIC (n=1)	↓Bile salts, bilirubin	Improvement in 1/1

**Only English language, nonduplicated publications measuring cytokines or chemokines in the blood of patients receiving MARS therapy were included. ↑ Increased; ↓ Decreased; ACLF Acute-on-chronic liver failure; AIH Autoimmune hepatitis; ALF Acute liver failure; ALT Alanine aminotransferase; ALP Alkaline phosphatase; AST Aspartate aminotransferase; BRIC Benign recurrent intrahepatic cholestasis; GGT Gamma-glutamyl transpeptidase; HBV Hepatitis B virus; HCV Hepatitis C virus; HEV Hepatitis E virus; HRS Hepatorenal syndrome; MARS Molecular adsorbent recirculating system; NASH Nonalcoholic steatohepatitis; OLT Orthotopic liver transplantation; PBC Primary biliary cirrhosis; PFIC Progressive familial intrahepatic cholestasis; PSC Primary sclerosing cholangitis; UK United Kingdom*

TABLE 5
Summary of publications on cholestatic pruritus and MARS therapy

Author (reference), year	**Country**	**MARS indication**	**n**	**Etiology**	**Cytokine outcome post-MARS**
Lisboa et al (present), 2012	Canada	Intractable pruritus	3	PBC (n=2), BRIC (n=1)	↓IL-1β, IL-2, IL-6, IL-8, IL-12 (p40), RANTES, TGF-α, TNF-α, TPO
Gay et al (44), 2011	Spain	Intractable pruritus	5	PBC (n=2), AIH (n=1), OLT graft rejection (n=1), Wilson disease (n=1)	CCL14, CCL15, PDGFA retained by MARS SAX
Novelli et al (45), 2011	Italy	ACLF + positive endotoxin activity assay	10	Ethanol (n=5), HCV (n=3), HBV (n=1), PBC (n=1)	↓IL-6, TNF-α
Wong et al (46), 2010	Canada	Type 1 HRS	6	Ethanol (n=4), NASH (n=1), HCV (n=1)	=IL-6, TNF-α
Novelli et al (47), 2009	Italy	ALF	45	Viral (n=21), other (n=19), unknown (n=5)	↓IL-6, TNF-α
Novelli et al (48), 2009	Italy	ALF	10	Ethanol (n=6), HBV (n=4)	↓IL-1, IL-6, TNF-α
Roth et al (49), 2009	Austria	ALF or ACLF	21	HCV (n=5), Wilson disease (n=3), trauma (n=2), AIH (n=1), PBC (n=1), toxic (n=1), unknown (n=3), other (n=5)	=MCP-1, IL-18
Ilonen et al (50), 2006	Finland	ALF or ACLF	81	ALF (n=49), ACLF (n=32)	↓IL-10=IL-6, IL-8, TNF-α, sIL-2Rα
Stadlbauer et al (51), 2006	Austria	ACLF	8	Ethanol (n=5), HCV (n=1), OLT graft dysfunction (n=1), other (n=1)	=IL-6, IL-8, IL-10, TNF-α despite demonstrable clearance
Isoniemi et al (52), 2005	Finland	ALF	49	Toxic (n=26), unknown (n=19), miscellaneous (n=4)	↓IL-10
Di Campli et al (53), 2005	Italy	ACLF	13	Ethanol (n=4), viral (n=5), NASH (n=1), Wilson disease (n=1), PSC (n=1), unknown (n=1)	↓IL-1β, IL-6, TNF-α in survivors
Auth et al (54), 2005	Germany	ALF	2	Wilson disease (n=2)	↓IL-6, TNF-α ↑VEGF
Sen et al (55), 2004	UK	ACLF	18	Ethanol (n=15), ethanol + HCV (n=3)	=IL-6, IL-8, TNF-α
Luo et al (56), 2003	China	MODS	1	Severe acute respiratory syndrome (n=1)	↓IL-6, IL-8, TNF-α
Ambrosino et al (57), 2003	Italy	ACLF	17	(n=17)	↑IL-6 ↓TNF-α
Guo et al (58), 2003	China	ALF or ACLF	24	HBV (n=17), ethanol (n=3), drug-induced (n=3), HEV (n=1)	↓IL-4, IL-6, IL-8, TNF-α, IFN-γ

Only English language, non-duplicated publications having cholestatic pruritus as the indication for MARS therapy were included. ↑ Increased; ↓ Decreased; = Unchanged; ACLF Acute-on-chronic liver failure; AIH Autoimmune hepatitis; ALF Acute liver failure; ALP Alkaline phosphatase; ALT Alanine transaminase; AST Aspartate transaminase; BRIC Benign recurrent intrahepatic cholestasis; CCL Chemokine (C-C motif) ligand; GGT Gamma glutamyl transpeptidase; HBV Hepatitis B virus; HCV Hepatitis C virus; HEV Hepatitis E virus; HRS Hepatorenal syndrome; IL Interleukin; IFN Interferon; MARS Molecular adsorbent recirculating system; MCP Monocyte chemotactic protein; MODS Multiple organ dysfunction syndrome; NASH Nonalcoholic steatohepatitis; OLT Orthotopic liver transplantation; PDGFA Platelet-derived growth factor A; PBC Primary biliary cirrhosis; PFIC3 Progressive familial intrahepatic cholestasis type 3; RANTES Regulated on activation, normal T cell expressed and secreted; SAX Strong anion exchange; TGF Transforming growth factor; TNF Tumour necrosis factor; TPO Thrombopoietin; UK United Kingdom; VEGF Vascular endothelial growth factor

hyporesponsiveness (24). The mRNA expression of the acute-phase protein SAA1 was also increased during therapy (mean fold-change 2.35), which can induce IL-10 secretion by neutrophils (25). EFNA2 expression is augmented after MARS, also being an inhibitor of T cell chemotaxis (26) and apoptosis (27). Among the downregulated genes was *TRIL*, an innate immunity gene involved in the response to bacterial lipopolysaccharide. Its knockdown in human peripheral blood mononuclear cells has been shown to attenuate lipopolysaccharide signalling through TLR4 thereby affecting cytokine production (28). EPHB1 (Ephrin receptor B1 – 2.26-fold decrease in expression post-MARS) has been shown to be elevated in the blood and in exudate lymphocytes in synovial tissues from patients with rheumatoid arthritis (29). Taken together, the changes in protein mediators and gene transcripts appear to suggest a potential immunomodulatory effect associated with MARS therapy.

Patients with pruritus show a significantly higher activity of autotaxin, an enzyme that converts lysophosphatidylcholine into lysophosphatidic acid, when compared with their control counterparts (14). MARS was associated with reduction of activity of autotaxin in two of three patients in the present series, while one patient achieved comparable symptomatic improvement despite a further increase in the activity of this enzyme, albeit from much lower baseline levels.

Our study was conceived as an exploratory pilot study to test the the feasibility of detecting circulating cytokines/chemokines and potentially meaningful changes in gene expression in the blood of cholestasis patients with intractable pruritus and it was performed before the intensification of referrals of cholestatic pruritus patients for therapy at our centre. A number of limitations, such as the number of patients, the lack of characterization of filtered substances in the dialysate and the determination of cell types responsible for the mRNA transcription patterns found, are acknowledged and remain to be addressed in future studies.

SUMMARY

We conclude that MARS therapy is associated with acute changes in cytokine/chemokine levels concurrent with changes in gene expression in blood cells. It is conceivable that the clinical benefit of such therapy in cholestasis patients with intractable pruritus may not exclusively result from filtration of pruritogens but also from systemic changes in cytokine and chemokine levels, and to those ascertained in blood cells at the transcriptome level.

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