AUTHOR'S REPLY

We acknowledge and express thanks to the authors for reviewing our study and bringing out relevant points for discussion. We totally agree that the prevalence of 100% vitamin K deficiency at the time of enrollment (*i.e.* at 7th day of antibiotic therapy) can be attributed to prematurity and co-existing sepsis for which the neonates required antibiotic therapy. We collected data on type of antibiotics in the study population as shown in ***Table* I**.

We did not find any specific class of antibiotics, which led to the vitamin K deficiency as evident by PIVKA levels >2 ng/mL. All the babies who had any episode of clinical bleed before enrollment were excluded. Regarding the postnatal age of 10.5 and 10 days in both the groups, we enrolled babies at 7th day of antibiotic therapy and babies with both early and late onset sepsis were enrolled. Neonatal cholestasis was one of the exclusion criteria at the time of enrollment.

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TABLE I DISTRIBUTION OF ANTIBIOTIC THERAPY BETWEEN THE TWO GROUPS OF NEONATES WITH SEPSIS

Antibiotic	*Vitamin K group (n=41)*	*Control group (n=39)*
Ciprofloxacin	20 (48.7)	15 (36.5)
Amikacin	41 (100)	39 (100)
Piperacillin-Tazobactam*	20 (48.7)	28 (71.2)
Vancomycin	7 (17.1)	6 (15.4)
Cefoperazone- Sulbactam	2 (4.8)	5 (12.8)
Metronidazole	0	1 (2.5)
Amoxycillin-clavulanate	3 (7.3)	2 (5.1)
Ampicillin	2 (4.8)	0
Netilmicin	2 (4.8)	1 (2.5)
Cefazolin	1 (2.4)	0
Meropenem	1 (2.4)	1 (2.5)
Cefotaxime	0	1 (2.5)

*Data presented as n (%); all P>0.05 except *P=0.02.*

Multi-use Hypertonic Saline Packets for Nebulization – A Threat for Patients with Cystic Fibrosis in India

Hypertonic saline is used for nebulization in various respiratory conditions – both in adults and children. Patients with Cystic fibrosis (CF) are required to use it many times a day for airway clearance to hydrate the viscid mucus in their airways [1].

In India, the only formulation of hypertonic saline available is a 3% solution dispensed in 100 mL sterile packs/bottles. On an average, each CF patient reuses the same bottle costing approximately Rs.100 for 5 to 6 days. This leads to a unique problem of contamination of the solution with bacteria, including *P. aeruginosa*, the very organism against which much of the antibiotic treatment is directed at, in CF patients.

Pseudomonas is ubiquitous in the environment and thrives on wet surfaces. Lack of adequate microbial clearance and pro-inflammatory environment, which are characteristic of CF airways, sets the stage for a downhill course once it colonizes the CF airways. In one study, the 8-year risk of death was found to be 2.6 times higher in patients colonized with *Pseudomonas* than in those without [2]. Isolation of *P. aeruginosa* from the airway secretions of a 2-month-old baby, 3 weeks after initiation of hypertonic saline nebulization prompted us to check the nebulization solution for bacterial contamination.

We performed surveillance cultures on 12 selected samples of hypertonic saline drawn from the bottles/ packs being reused by CF patients. Eight of the 12 samples (66%) were contaminated, 4 growing multiple bacteria. *Pseudomonas* strains isolated were *P. aeruginosa*, *P. putida* and *P. stuzeri*; one isolate from each of the three contaminated samples. Other bacteria were non-fermenting Gram negative bacilli (other than *Pseudomonas* and *Acinetobacter*), *Klebsiella*, *Citrobacter diversus*, *Acinetobacter haemolyicus* and coagulase-negative *Staphylococcus*.

Contaminated hypertonic saline solution can be a source of infection not only for CF patients, but also for those whose airway defenses are altered due to other reasons. To overcome this problem, we now pack 150 mg of pharmaceutical grade sodium chloride powder, which the caregiver can dilute with commercially available 'sterile water for injection' dispensed in 5 mL packs. We tested this

freshly-mixed solution to be 3% NaCl and bacteriologically sterile. Individually weighing and packing sodium chloride salt imposes a high workload on our pharmacy service and may be difficult to replicate. However, it is an interim arrangement that can be practiced till smaller single use 5- to 10 mL aliquots of hypertonic saline are made commercially available in India at affordable costs.

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Significance of Variation of Uncertain Significance: A Clinician's Dilemma

With completion of Human genome project, focus has now shifted to genetic level of disease etiology, outcome and prognosis; as a result, there has been an upsurge of genetic testing worldwide. American College of Medical Genetics and Genomics (ACMG) classifies any variant in to five categories described as pathogenic, likely pathogenic, uncertain significance, likely benign, and benign [1].Variation of unknown significance (VUS) falls in the grey zone. It has aptly been described as genetic purgatory [2] where uncertainty about the test results has been compared to purgatory for patients. Additionally, unnecessary actions/inactions on the basis of VUS report has led to inappropriate interventions and medical lawsuits against the clinician [3]. Hence fellow pediatricians and subspecialty experts, who order such genetic tests, should know about VUS, its implications for their patients and for themselves.

Dilemmas such as how should the patients be counseled, what follow-up studies should be done, what happens when a variant is reclassified, often arise. These questions are troublesome enough for geneticists, but are even more challenging for clinicians without specialist training in genetics, who are increasingly encountering genetic test results in office practice. We need to explain to parents, before ordering a test, that there is a probability of getting a positive, negative and may be an inconclusive result also. American College of Medical Genetics and Genomics guidelines in 2015 mention that a VUS report should not be a basis for further interventions [1]. There are reports of overtreatment where preventive implantable cardioverter and defibrillator was implanted in a whole family on the basis of VUS report for a chanellopathy [2], and preventive mastectomy done in patients with *BRCA 1* gene positive VUS report [4]. On the other hand, there has been a law suit filed for no action taken on a VUS reported *SCN1A* mutation in a child with Dravet syndrome wherein the variant was later reclassified as pathogenic variant and child died later and received antiepileptic drug that is contraindicated in such patients [3]. These cases bring to the forefront the potential importance of how VUS results are interpreted and their implications on patients and clinicians.

The current approach to VUS seems like passing the buck in a poker game, from the laboratory to the clinician on to the geneticist, and back to clinician most of the times. This further stresses the need for basic genetics education among non-genetics professionals, and it is vital that this education includes information on VUS to avoid the consequences of overtreatment and mismanagement. At the same time, for the patient and family's benefit, one visit to clinical geneticist is important for proper evaluation and accurate genetic counseling.

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