A Review of the Clinical Characteristics, Management and Outcomes of Neonates Exposed to Meconium Stained Amniotic Fluid at Chris Hani Baragwanath Academic Hospital

A research report submitted to the Faculty of Health Sciences for the Degree of Master of Medicine in Paediatrics

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Declaration

I, Masida Linda Ali-Dikole, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in Paediatrics at The University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

11th of November 2022 in Orange Grove, Johannesburg.

Dedication

To my loving and supporting family, thank you.

Presentations arising from this research project

The findings of this project have been presented to:

- University of Witwatersrand Research Day 30th September 2021
- Department of Paediatrics Research Day 02nd December 2021

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Contents

Abbreviations

BPD: Bronchopulmonary dysplasia
CHBAH: Chris Hani Baragwanath Academic Hospital
CLD: Chronic lung disease
CMV: Conventional mechanical ventilation
CSF: Cerebrospinal fluid
HFOV: High frequency oscillatory ventilation
HIC: High income countries
HIE: Hypoxic ischaemic encephalopathy
ILCOR: International liaison committee on resuscitation
IVH: Intraventricular haemorrhage
LMIC: Low to middle income countries
MAS: Meconium aspiration syndrome
MSAF: Meconium stained amniotic fluid
NPO2: Nasal prong oxygen
NCPAP: Nasal Continuous Positive Airway Pressure
NICU: Neonatal intensive care unit
PPHN: Persistent Pulmonary Hypertension of the Newborn
PPV: Positive pressure ventilation
TTN: Transient tachypnoea of the newborn
VACTERL: Vertebral, Anorectal, Cardiac, Trachea-esophageal, Renal, Limbs Association

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Introduction

During intrauterine life, amniotic fluid surrounds the foetus and plays multiple roles essential to foetal well-being (1) . It is for the foetus, a complex and dynamic environment with protective and nutritive functions that changes as pregnancy progresses (2). This milieu at times becomes stained with meconium prior to or during labour, with reported incidences of meconium stained amniotic fluid (MSAF) of 5% in preterm gestations (30 to37 weeks), 15-20% in term gestations (37- 41 weeks) and 23-52% in post-term pregnancies (> 41 weeks) (3–6). The mechanism of meconium passage remains a subject of debate, between mainly physiological maturation of the gastrointestinal tract and evidence of foetal compromise . Causes implicated in the passage of meconium in utero include: maternal factors, such as substances ingested (e.g. Misoprostol, castor oil, herbal remedies), hypertension, intra-uterine infections, pregnancy induced cholestasis; and foetal factors such as hypoxia, and cord compression resulting in increased gastrointestinal peristalsis and anal sphincter relaxation through the stimulation of the vagus nerve (3,9–15).

The management of neonates exposed to MSAF has evolved with time guided by the growing understanding of the pathophysiology of meconium aspiration syndrome (MAS) as well as asphyxia . Research has shown no benefits to suctioning vigorous neonates exposed to MSAF (7,16). In addition, there is currently no evidence for routine suctioning of non-vigorous neonates unless there is a strong suggestion of tracheal obstruction (17). The normal steps of neonatal resuscitation should be followed. The focus has essentially shifted from removing meconium, to helping neonates breathe as early as possible to avoid complications associated with hypoxia (17). Following initial stabilization, all neonates exposed to MSAF should be observed for at least 24 hours and those with signs of respiratory distress should be admitted, ideally into a neonatal intensive care unit (NICU) setting, with further management based on their clinical picture with anticipation of rapid deterioration in the event of disease progression (18).

MSAF can have deleterious effects on the neonate with short and long-term consequences (9). Some short term complications of MSAF include: injury to the umbilical cord vessels, MAS and its complications, including NICU admissions, neonatal sepsis, poor APGAR scores with increased need for resuscitation, perinatal asphyxia, seizures, and grade 3-4 intra-ventricular haemorrhage (IVH) (4–6,13,15,19–21). Reported long-term complications associated with

MSAF, which are mainly secondary to MAS, include: chronic lung disease (CLD) , reactive airway disease, decrease in lung function, cerebral palsy, autism spectrum disorder, seizures and neurodevelopmental impairment (5,6,12–14,22–26). A number of studies have also reported an increased rate of perinatal death in neonates exposed to MSAF, with reported mortality rates as high as 46.9% (3,20,26–30). MSAF is graded as thin (grade 1), moderate (grade 2) or thick (grade 3). Thin MSAF refers to a light green or yellow stain from a small amount of meconium in a plentiful quantity of the amniotic fluid, moderate describes where there is fair quantity of amniotic fluid with a resultant 'khaki green' or brownish colour and thick if particulate matter was observed in the amniotic fluid, that may be reduced in volume (3,27,31–33). Thick meconium has been associated with complications including higher frequency of non-reassuring cardiotocography and instrumental deliveries, lower 5 minute APGAR, increased risk of respiratory distress, MAS, HIE, septicemia, NICU admissions as well as mortality (7,10,20,26–28,30–32,34–37). Apart from the use of amnioinfusion in low resource settings, the management of neonates born through thin or thick MSAF is identical (10,17)

Meconium aspiration syndrome is one of the most anticipated neonatal complications of MSAF, and is defined as a clinical triad of MSAF, respiratory distress, and typical radiological features (overexpansion of the lungs with widespread coarse, patchy infiltrates, occasional air leaks) (13,18). It is a multifactorial disease with various pathophysiological processes (38). These processes include airway obstruction, inactivation of surfactant and chemical pneumonitis, amongst others (39). The incidence of MAS varies across different settings where access to healthcare, level of expertise and overall resource availability differ. In developed countries rates of MAS have been reported to be between 0.2% and 5% (6,12,18,21,38). There is limited data on the incidence in developing countries, with reported rates varying from 2-14% (3,40). Complications associated with MAS include air leaks, persistent pulmonary hypertension of the newborn (PPHN), hypoxic ischaemic encephalopathy (HIE), shock, myocardial dysfunction and chronic lung disease (CLD) in term neonates (19,22). Death is an important adverse outcome in patients affected by MAS, with mortality rates reported between 1.2 to 32% (5,8,12,19,24,29,35,41).

At Chris Hani Baragwanath Academic Hospital (CHBAH), MSAF exposure is not an uncommon reason for referral of neonates to the neonatal unit for initial assessment and further management. This study aimed to describe the prevalence, clinical characteristics,

management, and outcomes of neonates born through MSAF in a tertiary level, public hospital in Johannesburg, South Africa.

Materials and Methods

Study design and study population

This was a retrospective descriptive study of neonates born at, or referred to the neonatal unit at CHBAH, with exposure to MSAF, from the 1st of January 2018 to the 30th of June 2018.

Inclusion criteria:

I. All neonates born at CHBAH, who were exposed to MSAF and referred to the neonatal unit at CHBAH immediately post-delivery, for initial assessment and management.
II. All neonates born in surrounding clinics, who were exposed to MSAF and referred to the neonatal unit at CHBAH immediately post-delivery, for initial assessment and management.

Exclusion criteria:

I. Neonates born through MSAF who were referred from other hospitals directly to the neonatal intensive care unit (NICU) for ventilation were excluded. These babies were excluded as their initial assessment, management and admission would have occurred at the referring hospital and multiple variables on initial assessment and management would not have been available for analysis. In addition, data on number of live births during the study period from these referring hospitals are not available for calculating the incidence of MSAF and MAS.

Study setting

The study was conducted in the neonatal unit at Chris Hani Baragwanath Academic Hospital (CHBAH) a public, tertiary hospital located in the outskirts of Soweto in Johannesburg, South Africa. It is a referral center for clinics and maternity obstetric units in its drainage area for high-risk mothers and neonates, including all neonates born through MSAF. The hospital conducts approximately 20000 of the 33000 annual deliveries in Soweto. Chris Hani Baragwanath Academic Hospital is also a referral hospital for primary and secondary level hospitals in and around Johannesburg for neonates requiring tertiary care, where resources for such care may not be available.

Standard hospital protocol in the management of infants exposed to MSAF

All high-risk neonates born at CHBAH or referred to CHBAH from surrounding clinics are assessed in a triage nursery for further management. At CHBAH recommendations from the International Liaison Committee on Resuscitation (ILCOR) are followed for neonates requiring resuscitation, whereby intratracheal suctioning for non-vigorous infants is no longer routinely conducted (17). The aim is to establish ventilation in the first minute of life. Therefore, intubation and intratracheal suctioning will only be performed if the airway is suspected to be obstructed by meconium (17). Neonates born through MSAF, either born before arrival and those born in clinics around CHBAH are all referred to CHBAH for assessment even if assessed to be clinically well. Those who require resuscitation at birth will be resuscitated and stabilized prior to referral. Intratracheal suctioning of non-vigorous neonates is not performed. Their resuscitation practice is limited to manual ventilation using a bag and mask and cardiopulmonary resuscitation. If intubation is required for transport, it would be performed by the advance life care paramedic team prior to transfer. At CHBAH, following stabilization and assessment of both inborn and outborn MSAF exposed neonates, neonates who are well are transferred to their mothers in the post-natal ward and reassessed within 24 hours. Neonates exposed to MSAF who develop respiratory distress are managed with oxygen therapy and admitted for further management. Neonates in mild to moderate respiratory distress are managed with nasal cannula oxygen or nasal continuous positive airway pressure (NCPAP). Neonates who are in severe respiratory distress or who develop respiratory failure are intubated and ventilated in the NICU. Surfactant replacement therapy is considered in patients who require a fractional inspired oxygen concentration of more than 0.6 to maintain oxygen saturations above 90% or who develop PPHN, after obtaining a chest radiograph supporting the diagnosis of MAS and excluding air leaks. Neonates who develop PPHN are managed according to the unit protocol. This includes intubation and ventilation, with the goal of ventilation to maintain a pH of > 7.2, $PaCO_2$ of 45-60 mmHg and pre-ductal pulse oximetry saturations > 89% and post-ductal pulse oximetry saturations of > 70%. Adequate sedation and analgesia are prescribed as required, with minimal stimulation of the infant. Normal mean arterial pressures (MAP) are targeted with use of inotropes if hypotensive. High frequency oscillatory ventilation (HFOV) is started if indicated. Treatments to reduce pulmonary artery pressures include sildenafil and inhaled nitric oxide, if the oxygenation index is above 25 and after an echocardiogram has confirmed the diagnosis of PPHN and ruled out cyanotic congenital heart disease.

Study definitions

For this study, the following definitions were used: Meconium stained amniotic fluid (MSAF) refers to greenish staining of amniotic fluid with meconium after its passage by the foetus in utero (5). Foetal distress describes compromise of the foetus inferred from an abnormal cardiotocography tracing, or foetal tachycardia or bradycardia. Meconium aspiration syndrome is defined as a clinical syndrome in which a neonate delivered through MSAF develops respiratory distress requiring oxygen therapy for more than 24 hours. A chest radiograph showing characteristic radiological findings may or may not be available. Mild respiratory distress refers to a neonate with tachypnoea but no other signs of respiratory distress. Moderate respiratory distress refers to a patient with tachypnoea with mild to moderate subcostal recessions. Severe respiratory distress refers to a patient with tachypnoea, moderate or severe subcostal recessions and/or grunting and cyanosis. Ventilation for MAS refers to a patient meeting the definition of MAS requiring ventilation within forty-eight hours of birth. Mild MAS refers to a need of supplemental oxygen at less than 40% for less than 48 hours; Moderate MAS with requirement of more than 40% oxygen and/or for more than 48 hours and Severe MAS if the neonate requires mechanical ventilation for more than 48 hours (13). Persistent pulmonary hypertension of the newborn describes the persistence of high pulmonary vascular resistance resulting in right to left shunting across the patent ductus arteriosus and/or foramen ovale (29). This may be diagnosed clinically with differential saturations between the right arm (pre-ductal saturations) and the rest of the body (post-ductal saturations) of more than 10% or may be diagnosed on echocardiography. HIE was diagnosed if the following criteria were met: Apgar score of 5 or less at 10 minutes; arterial blood gas taken within an hour after birth showing a pH of less than 7 and/or a base deficit of more than 12; evidence of encephalopathy; evidence of multi-organ dysfunction (42). It is further classified into HIE stage I, II, and III according to the modified Sarnat and Sarnat classification (43). Air leak syndromes refers to the presence of pneumothorax, pneumomediastinum, pneumopericardium and pulmonary interstitial emphysema. Chronic Lung Disease (CLD) describes the need for supplemental oxygen for 28 days after birth (22). Case fatality rate refers to the neonates who die from complications attributable to MSAF within 7 days of birth, without any other attributable cause. Neonatal sepsis was defined as a positive blood or cerebrospinal fluid (CSF) culture with a significant pathogen. Early onset sepsis was defined as culture positive blood or CSF infection in the first 72 hours of life. Nosocomial sepsis was defined as culture positive blood or CSF infection after 72 hours of life in an admitted neonate.

Study procedures

Basic data on all neonates referred to the triage nursery for initial assessment and management are captured onto a research electronic data capture (REDCap) database (44). Entries on this REDCap database for all meconium exposed neonates during the study period were reviewed. All neonates who are assessed to be clinically unwell and admitted to the neonatal unit are captured onto a second REDCap database upon discharge or death. Entries on this REDCap database for neonates exposed to MSAF who required admission during the study period were also reviewed. Hospital records of patients with a diagnosis of MAS were retrieved for data collection and analysis.

Data collection

Data collected on all MSAF exposed neonates included: Maternal and neonatal demographics, antenatal care, maternal co-morbidities, suspicion of chorioamnionitis, antibiotics given during labour, details of delivery and resuscitation, initial blood gas parameters, initial maximum mode of respiratory support and outcome, including admission diagnosis, if admitted. Further data collected for neonates admitted for MAS included: highest mode of respiratory support, laboratory results suggestive of neonatal sepsis (elevated C reactive protein of >10mg/L, white cell count >30 or <9 $x10^9$/L , platelet count <150 x 10^9/L) as well as positive blood or CSF culture , in-hospital management including need and type of inotropic support, the administration of surfactant, sildenafil and nitric oxide, development of complications, including PPHN, HIE, air leak syndromes and CLD. The outcome measures reviewed included discharge from hospital, transfer to another facility or death.

Statistical analysis

Data was captured onto an Excel database and analyzed using Statistica, version 12. Means and standard deviations were used to describe continuous variables with normal distribution. Median and ranges were used to describe continuous variables that were not normally distributed. Frequencies and proportions were used to describe categorical variables. To compare MSAF exposed neonates who developed MAS to those who did not the Student t-test was used when comparing parametric data. The Mann-Whitney U test was used to compare non-parametric data and the Pearson Chi-square test to compare categorical variables. Differences between the two groups were considered statistically significant when the p-value was less than 0.05.

Results

During the study period, there were a total of 9239 live births at CHBAH and 4448 in the surrounding clinics (Figure 1). Of these, 598 neonates were born through MSAF, giving an incidence of 44/1000 live births. Baseline maternal and neonatal demographic characteristics of neonates born through MSAF are shown in Table 1. Approximately 98.5% of mothers had booked at a healthcare facility antenatally. Majority (93.9%) of deliveries were inborn. The median maternal age was 27 years. Human Immunodeficiency Virus positive status was seen in 28% of mothers. Caesarian section was the most common mode of delivery (51.7%), of which 10.9% were performed electively and 89.1% as an emergency. The most common indication for emergency caesarean section was foetal distress (75.7%). Male sex predominated at 53.8%. The median birth weight was 3125g with a median gestational age of 39 weeks. The median APGAR score was 8 and 9 at 1 and 5 minutes respectively.

Postnatal management of neonates born through MSAF (Table 2)

Information on initial resuscitation was available for 494 neonates exposed to MSAF. Following delivery, 48.4% (n=239) of neonates required resuscitation; positive pressure ventilation (239; 48.4%); chest compressions (27; 5.5%); intubation (24; 4.9%) and adrenaline (5; 1%). Details on suctioning was available for 191 patients, of which 23.6% of patients were suctioned prior to positive pressure ventilation (95.6 % oro-nasal and 4.4% endotracheal) and 10% were suctioned after positive pressure ventilation (58% oro-nasal and 42% endotracheal). Initial need for respiratory support, following stabilization, was recorded for only 336 of the 598 MSAF exposed patients and included no respiratory support (9; 2.7%), nasal prong oxygen (285; 84.8%), NCPAP (5; 1.5%), conventional mechanical ventilation (CMV) (28; 8.3%) and HFOV (9; 2.7%). Blood gas parameters collected within the first hour of life were recorded for 89 patients. The median pH was 7.3, median partial pressure of carbon dioxide (pCO2) was 32.9 mmHg, median partial pressure of oxygen (pO2) was 98.1 mmHg, median base deficit was 10 mmol/L and median lactate was 7.1 mmol/L.

Of the neonates born through MSAF, immediate outcome was known in 580 patients as 18 patients had no documented outcome. Among these, admission either to the intensive care unit (level III nursery), the high care ward (level II nursery) or the general neonatal ward (level I nursery) was required in 55.5% (n=322/580) (Figure 1). Two hundred and fifty-two neonates (252/580; 43.4%) did not require admission (189 neonates (32.6%) were transferred to the

postnatal ward to room in with the mother for review the next day, and 63 neonates (10.9%) were transferred to the postnatal ward to room in with the mother and not for further review). None of the neonates in the postnatal wards who were reviewed the following day required subsequent admission for respiratory conditions. Six (6/580; 1.0%) neonates demised in the immediate post-partum period, within 6 hours of life: 2 HIE, 1 MAS, 1 MAS with HIE, 1 with congenital abnormalities (VACTERL association not considered to be life threatening) and 1 with unknown cause of death. Indications for admission in patients with MSAF (Figure 1)
Of the 322 neonates that required admission, admission diagnosis was only documented in 227 cases. Among these, the most common indications for admission were for respiratory diagnoses, in 87.2% (n=198) of cases. These included transient tachypnoea of the newborn (TTN) (52; 22.9%), MAS (135; 59.5%) and congenital pneumonia (11; 4.8%). Thirty-seven (16.3% of 227) patients were admitted with HIE. After excluding all the patients with unknown data (18 unknown data on immediate outcome and 95 unknown admission diagnosis), there were 485 MSAF exposed neonates with known outcome and diagnosis. HIE accounted for 7.6% of these patients. Hypoxic ischaemic encephalopathy was graded as I, II, III in 58.8%, 20.6% and 20.6% of patients respectively.

Meconium aspiration syndrome

A diagnosis of MAS was made in 135 (27.8%) of the 485 MSAF exposed neonates with known immediate outcome and diagnosis, giving an incidence of 10/1000 live births. Comparing neonates born through MSAF who developed MAS (n=135) to those who did not (n=350), the neonates with MAS were more likely to have lower 1- and 5-minute APGAR scores, metabolic acidosis, require any resuscitation at delivery and be diagnosed with HIE (Table 3). Seventy five of the 135 hospital records of patients admitted with MAS were retrieved for review of management and outcomes (Table 4). Twenty-four (32%) of the 75 patients admitted with MAS required invasive ventilation. Management in patients included surfactant therapy in 10 (13.3%), sildenafil in 10 (13.3%); inotropic support in 11(14.7%), and inhaled nitric oxide in 4 (5.3%). PPHN was diagnosed in 13 (17.3%) patients and pulmonary air leaks in the form of pneumothorax were seen in 4 (5.3%) patients. The median duration of ventilation was 4 days. A diagnosis of CLD was made in 4 patients. All 4 patients were successfully weaned off supplemental oxygen prior to discharge. Median length of hospital-stay in patients admitted with MAS was 8 days.

Mortality in neonates exposed to MSAF and MAS

Of the 485 neonates, 12 demised (6 in the immediate postpartum period, within the first 6 hours of life, and 6 admitted neonates), giving an all-cause mortality rate, in neonates exposed to MSAF, of 2.5%. The causes of death were unknown for 1(8.3%), asphyxia related 6 (50%), early neonatal sepsis 1 (8.3%), nosocomial sepsis 1(8.3%), meconium aspiration 2 (16.7%) and congenital abnormalities 1 (8.3%). Of the 75 patients reviewed with MAS, 4 died within the first 7 days, giving a case fatality rate of 5.3%. All-cause mortality in patients admitted with MAS was 9.3% (n =7). Of the 7 neonates that demised, 1 died in the immediate post-partum period and all 7 were among those ventilated.

Discussion

The incidence of MSAF varies in literature. In high income countries (HIC) reported rates of MSAF range from 53 to 85.7 per 1000 live births in neonates born at ≥ 34 weeks gestation (45). In low to middle income countries (LMIC), MSAF was noted in 83 to 154 per 1000 live births (23,29,32,34,36). Though we expected to find a higher rate of MSAF, ours was lower at 4.4 % with an incidence of 44/1000 live births. The lower rates seen could be due to the improving antenatal and intrapartum care in our tertiary institution, as well as the decreasing rate of post-date pregnancies with high-risk mothers being scheduled for elective caesarian sections or induction of labor. However, incomplete entry of data of MSAF exposed neonates into the database, owing to the retrospective nature of the study could have resulted in underestimation of rates. Our rate was also lower compared to the 9% observed in a study conducted in New Delhi, India (30). Another possible explanation for the lower rates of MSAF seen in this study could be the failure of referral of well neonates born through MSAF from surrounding clinics, despite the existing protocol, as most of the MSAF exposed neonates required respiratory support with a minority of well babies observed in this subset.

Reviews in LMIC have reported caesarian section rates of 45.8% to 70.2% in pregnancies complicated by MSAF, of which up to 86.8% were emergency caesarian sections (19,26,34). A retrospective audit of mothers with MSAF conducted in a HIC reported on a caesarean section rate of 30.9% in Australian mothers and 27.8% in mothers born outside of Australia (46). Caesarean section as the mode of delivery in our study was 51.7 % of which 89.1% were emergency, which is comparable to rates in other LMIC but higher than that reported in the Australian study quoted above. One of the most common indications for emergency caesarian section is foetal compromise, a major risk factor for meconium passage in-utero.

In a prospective observational study conducted in Western India looking at perinatal outcomes of neonates born through MSAF, the authors noted that the majority of mothers were unbooked at 65%, with a mean age of 22 years, who were mostly primigravidae (62%)(26). This contrasts with our study, where most mothers received antenatal care, were slightly older, with a median age of 27 years, and were composed of fewer primigravidae. In our cohort, most neonates were of term gestational age, comparable to other studies that also reported mean term gestational ages of 37 to 39 weeks in their cohorts of MSAF exposed neonates (19,20,31,37,45).

A single center cohort study conducted in the United Kingdom post implementation of the 2015 changes in management of neonates born through MSAF, where routine endotracheal suctioning of non-vigorous infants is no longer recommended, noted an incidence of 22.7% of neonates born through MSAF (17,45). In their cohort, 7% required endotracheal suction post initial positive pressure ventilation (PPV), down from 94.4% prior to guideline changes. These infants met the description of non-vigorous with the criteria of being limp, having a heart rate of less than 100 beats per minute or not breathing , for which the policy was for routine suctioning as per the 2010 European resuscitation council prior to PPV (45). This was comparable to the 10% in our study that required suctioning post initial PPV. In accordance with recommendations from the 2015 ILCOR guidelines, the rates of intratracheal suctioning prior to PPV in our cohort was low at only 4.4%.

Neonates exposed to MSAF are reported to have increased need for resuscitation compared to controls, with reported resuscitation rates ranging between 7.3% and 31.5% (29,46). We observed that 48.4% of neonates required resuscitation. The passage of meconium in utero is a process often triggered by foetal hypoxia from a range of causes (47). This may result in a neurologically depressed neonate aspirating MSAF with a clinical presentation of HIE and meconium aspiration at the delivery (47). The presentation may include airway obstruction, requiring intubation and suctioning for improved oxygenation, chemical pneumonitis as well as surfactant de-activation resulting in respiratory failure (47). Thus, a higher proportion of neonates exposed to MSAF require resuscitation at birth compared to controls born through clear liquor.

Outcomes of neonates born through MSAF vary (20,31). In our study, nearly half of the neonates exposed to MSAF required admission, of which majority were for respiratory causes. Other studies conducted in LMIC noted a lower admission rate of 19.7% - 22% (3,26,27). A study comparing neonates born through clear amniotic fluid to neonates delivered through MSAF noted that 22.5% of neonates born through MSAF required admission versus 6.3% in those born through clear amniotic fluid (20). In LMIC in neonates exposed to MSAF, perinatal asphyxia rates range from 5.8-9%, which is higher than rates seen in non-MSAF exposed neonates of 2.4% (3,27,29). Our analysis noted that 7.6% of patients had HIE which was also a significant contributor to mortality with 50% of deaths being asphyxia related. In this study, of the 189 well MSAF exposed neonates who were transferred to their mothers for review in 24 hours, none required subsequent admission for respiratory distress. This could support discontinuation of the practice of reviewing all well MSAF exposed neonates in 24 hours.

The reported incidence of MAS ranges from 0.7-3.7 per 1000 live births in HIC and 1.9-6.6 per 1000 live births in LMIC (3,14,28,45,48). In an analysis conducted in South West Ethiopia, an incidence of 18.5% of MAS in infants born through MSAF was noted (34). In a study conducted in New Delhi, India, an overall rate of 7.5% of MAS was observed. In 2008, the incidence of severe MAS requiring ventilation at the same institution as our study was 1.43 per 1000 live births. Another study in South Africa noted a MAS rate of 12.2 per 1000 live births (49). We observed a higher rate of MAS of 27.8% and an incidence of 10 per 1000 live births. Our higher incidence could be attributed to over-diagnosing MAS as many patients did not have a chest X-ray done or documented, to radiologically support the diagnosis of MAS. The higher incidence may also be explained by the change in management related to intratracheal suctioning of non-vigorous neonates adopted in accordance with the ILCOR 2015 guidelines, as also reported in other studies (48)

One of the complications of MAS includes the need for mechanical ventilation (18). In 2008, in a review that analyzed neonates with severe MAS requiring ventilation at the same institution as our study, the incidence of ventilated neonates was 1.43 per 1000 live births, (50). In our cohort of patients diagnosed with MAS, the incidence of severe MAS requiring mechanical ventilation was slightly higher at 1.8 per 1000 live births. This higher incidence seen may be attributed to the change in resuscitation guidelines as mentioned previously. Of the 75 files reviewed, 13.3% of neonates required surfactant and sildenafil, and 14.7% required inotropes with 5.3% also requiring hydrocortisone for refractory hypotension. In the local study

quoted above, 24% of neonates ventilated with MAS developed air leaks, 57% developed PPHN, 14% received surfactant, 6% were treated with inhaled nitric oxide and 32% required HFOV (50). We found a much lower frequency of air leaks (5.3%), PPHN (17.3%) and infants requiring HFOV (9.3%). This rate of air leaks was more comparable to that noted in a cohort in a developed country of 8-12.9% (33). This could be explained by improved ventilation practices and surfactant therapy being more readily available for ventilated patients with MAS resulting in a decreased need for higher ventilatory support and decreased risk of air leaks. About 5% of survivors with MAS have been reported to still require oxygen at one month of age (47). We observed a similar rate of 5.3% of neonates with CLD in our cohort.

Mortality is a significant complication of MAS. Mortality in LMIC ranges from 2 to 20 % (3,19,27,51). An all-cause mortality rate in neonates exposed to MSAF of 2.5% was found in our study with a rate of 9.3% in the subset with MAS. MAS had a case fatality rate of 5.3%. These lower rates could be attributed to the fact that CHBAH being a tertiary care center, is able to provide conventional mechanical ventilation, surfactant therapy, HFOV and inhaled Nitric Oxide, to neonates with MAS, when indicated, therefore improving their chances of survival. A study in Pakistan reported a higher mortality rate of 19.4 % where similar resources were available, therefore our lower mortality rates cannot be explained purely by availability of resources but possibly other factors, such as better intrapartum and postnatal care (21).

Strengths of this study are that we were able to report on the clinical characteristics, management and outcome of a large sample of neonates exposed to MSAF. The analyses conducted on patients who were admitted were based on patient records giving a more accurate depiction of inpatient management. Limitations of the study include the retrospective nature of the study which affected data collection due to missing data. However, neonates with missing data were excluded from the analysis of variables. Chest X-rays were not done or reports not recorded in most cases, which could have also resulted in under or over-diagnosing MAS.

Conclusion

At CHBAH, there is a high incidence of neonates born through MSAF, and approximately half of these patients require resuscitation and admission. Meconium aspiration syndrome is a common respiratory complication of MSAF, further complicated by PPHN, increased rate of neonatal intensive care admission as well as mortality. Identification of high-risk mothers, expedition of delivery with presence of experienced clinicians for prompt assessment followed

by close monitoring and escalation of care should be prioritized for more favorable outcomes. In view of the high rate of MAS observed in this study compared to that previously reported in the same institution, further studies are warranted to assess the impact of the change in guidelines related to intratracheal suctioning of non-vigorous neonates on the incidence and outcomes of MAS.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics

This study was approved by the Human Research Ethics Committee of The University of The Witwatersrand, Clearance certificate no. M190220.

Availability of Data and Materials

Raw data is available to referees and to readers upon request.

Author Contributions

† This author contributed equally to this work and has first authorship. The two other authors contributed equally to work and shared senior authorship.

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Table 1:Characteristics of mothers with MSAF and neonates born through MSAF

Maternal Characteristics	
Age (years) (N=389) Median (IQR)	27 (23-32)
Parity (N=371) Median (IQR)	2 (1-3)
Gravidity (N=381); median (IQR) Primigravidae; n (%)	2 (1-3) 150 (39.4)
ANC (N=476) ; n (%) Yes No	 469 (98.5) 7 (1.5)
Race (N=595); n (%) Black Coloured White Indian	 573 (96.3) 18 (3) 2 (0.3) 2 (0.3)
Place of birth (N=590); n (%) Inborn Surrounding clinics Born before arrival	 554 (93.9) 32 (5.4) 4 (0.7)
Maternal co-morbidities; n (%) HIV (N=579) Positive On treatment Negative Hypertension (N=531) Diabetes (N=595) Tuberculosis (N=592) Suspicion of Chorioamnionitis (N=499) Intrapartum antibiotics (N=485)	 162 (28.0) 109 (67.2) 417 (72.0) 17 (3.2) 1 (0.2) 1 (0.2) 9 (1.8) 17 (3.5)

Neonatal Characteristics	
BW (g) (N=588) Median (IQR)	3125 (2825-3495)
GA (weeks) (N=404); median (IQR)	39 (38-40)
Preterm (34-36); n (%)	39 (9.6)
Term (37-41); n (%)	351 (86.8)
Post term (>41); n (%)	13 (3.2)
Sex (N=556); n (%)	
M	299 (53.8)
F	257 (46.2)
Mode of delivery (N=578); n (%)	
NVD	263 (45.5)
C/S	299 (51.7)
Vacuum	9 (1.6)
Forceps	2 (0.3)
Breech	5 (0.9)
APGAR (median, IQR)	
1 min (N=576)	8 (6-9)
5 min (N=573)	9 (9-10)
10 min (N=137)	10 (9-10)

For each variable numbers in brackets represent number of patients with known data analyzed.

ANC: antenatal care; HIV: human immunodeficiency virus; BW: birth weight; GA: gestational age; M: male; F: female; NVD: normal vaginal delivery; C/S: caesarean section.

Table 2: Postnatal stabilization and complications in infants born through MSAF

	N (%)
Any Resuscitation (N=494); n(%) PPV only PPV and CPR Intubation CPR and Adrenaline	239 (48.4) 239 (48.4) 27 (5.5) 24 (4.9) 5 (1.0)
Blood gas parameters (N=89); median (IQR)	
pH (N=89)	7.3 (7.1-7.4)
$PaCO_2$ (mmHg) (N=88)	32.9 (27-40)
PaO_2 (mmHg) (N=86)	98.1 (63.5-124)
BE (mmol/L) (N=87)	-10 (-16.2-(-5.8))
Lactate (mmol/L) (N=72)	7.1(4.3-11)
Initial Respiratory support within 48 hours (N=336); n (%) Room air Nasal canula CPAP CMV HFOV	 9 (2.7) 285 (84.8) 5 (1.5) 28 (8.3) 9 (2.7)
MAS (N=485); n (%)	135 (27.8)
NICU (N=485) ; n (%)	39 (8)
Staging of HIE (N=485); n(%) HIE 1 HIE 2 HIE 3	 23 (4.7) 7 (1.4) 7 (1.4)
Leukocytosis/ leucopenia (N=184); n (%)	21 (11.4)
Thrombocytopenia (N=185); n (%)	9 (4.9)
CRP (mg/L) (N=147); n (%) >10 <10	 60 (40.8) 87 (59.2)
ENNS (N=227); n (%)	3 (1.3)

NOS (N=227); n (%)	5 (2.2)
CLD (N=485); n (%)	4 (0.8)
Total admissions (N=580); n (%) NICU (N=580); n (%)	227 (39.1) 39 (6.7)
Transferred to mother and no further review (N=580); n (%)	63 (10.9)
Transferred to mother but reviewed the next day (N=580); n (%)	189 (32.6)
Discharged (N=227); n (%)	217 (95.6)
Mortality (N=485); n (%) Immediate post-partum period NICU Survivors; n (%)	12 (2.5) 6 (1.2) 6 (1.2) 473 (97.5)

For each variable numbers in brackets represent number of patients with known data analyzed; PPV: positive pressure ventilation manually with either bag-mask-valve or T-piece resuscitator; CPR: cardiopulmonary resuscitation; $PaCO_2$: Partial pressure of carbon dioxide; PaO_2: Partial pressure of Oxygen; BE: base excess ; Leukocytosis: white cell count >30 $x10^9$/L; Leukopenia <9 $x10^9$/L, Thrombocytopenia: platelet count <150 x 10^9/L; CRP: C-reactive protein; CPAP: Continous Positive Airway Pressure ; NICU: neonatal intensive care unit; CPAP: continous positive airway pressure ; CMV: conventional mechanical ventilation ; HFOV: high-frequency oscillatory ventilation ; MAS: meconium aspiration syndrome**;** ENNS: early onset neonatal sepsis ; NOS: nosocomial sepsis; CLD : chronic lung disease

Table 3: Comparison of neonates with MAS to those without MAS

Variables	MAS (N = 135)	No MAS (N = 350)	P-value; OR (CI)
1 min APGAR; median (IQR)	7 (5-8)	8 (7-9)	0.011
5 min APGAR; median (IQR)	9 (8-10)	10 (9-10)	0.001
Initial Blood Gas			
pH; median (IQR)	7.2 (7.1-7.4)	7.3 (7.2-7.4)	0.021
Base deficit; mean (SD)	12.3 (7.7)	9.7 (3.9-14.8)	0.007
Lactate; median (IQR)	8 (5.6-13.9)	5.6 (3.2-8.1)	0.004
Need for resuscitation; n (%)	77 (57.0)	162 (34.9)	< 0.001; 2 (1.6 – 3.6)
PPHN; n (%)	16 (11.8)	0 (0)	0.011
HIE; n (%)	28 (20.7)	12 (2.6)	0.014

p-value <0.05 clinically significant; MAS: meconium aspiration syndrome; OR: odds ratio; PPHN: Persistent Pulmonary Hypertension of the Newborn; HIE: hypoxic ischaemic encephalopathy; IQR: interquartile range; standard deviation.

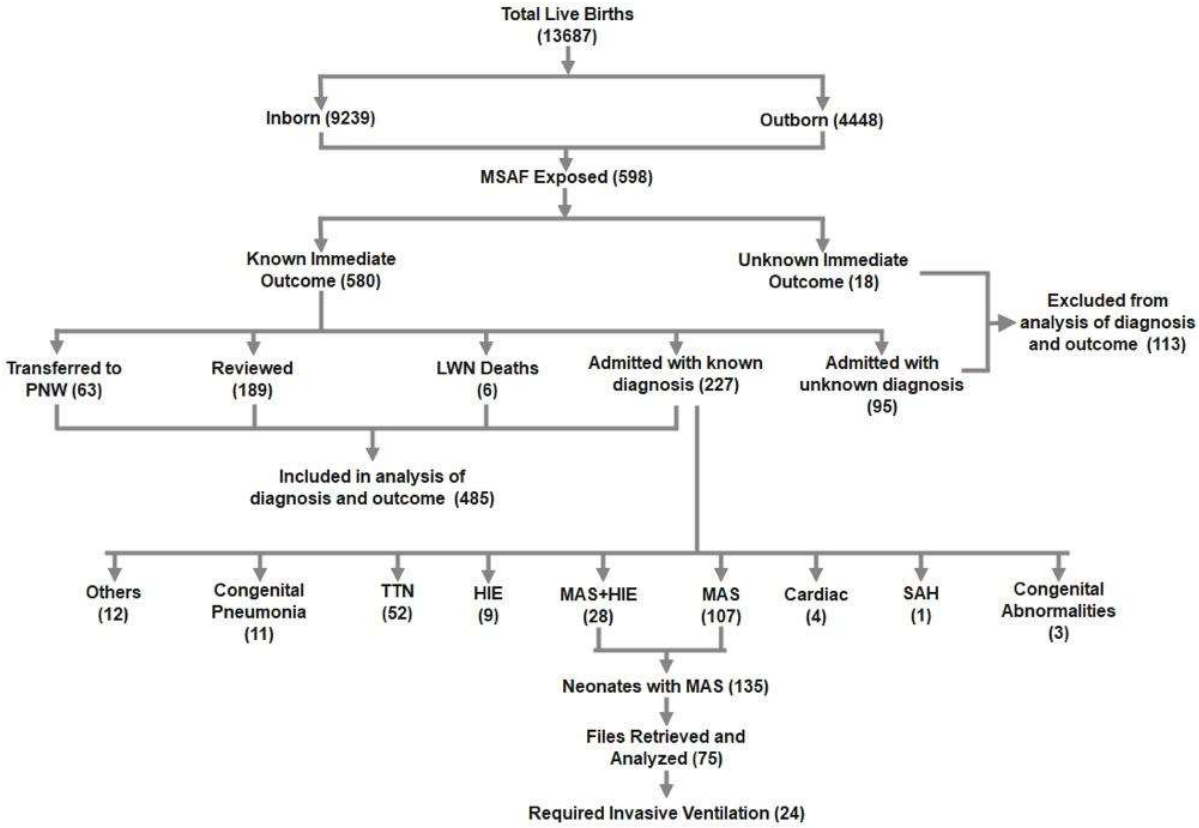
Table 4: Management and complications of neonates admitted with MAS

Data in retrieved records (N=75)	N (%)
Highest level of respiratory support Nasal cannula NCPAP CMV HFOV	 46 (61.3) 5 (6.7) 17 (22.7) 7 (9.3)
Inotropic support	11(14.7)
Surfactant	10 (13.3)
Sildenafil	10 (13.3)
Inhaled Nitric Oxide	4 (5.3)
PPHN Clinically Clinically and echocardiogram	13 (17.3) 3 (23) 8 (61.5)
Airleaks Pneumothorax Intercostal drain	 4 (5.3) 4 (5.3)
CLD	4 (5.3)
HIE Therapeutic hypothermia Seizures	24 (32) 6 (8) 9 (12)
Deaths Survived to discharge	7 (9.3) 68 (90.7)

Ventilatory support includes non-invasive and invasive support

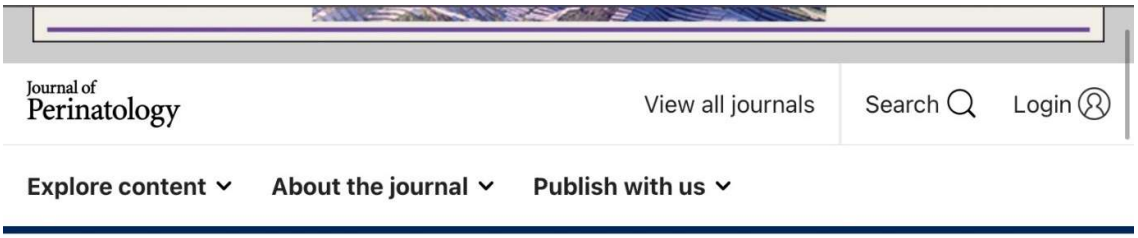
NCPAP: nasal continuous positive airway pressure; CMV: conventional mechanical ventilation; HFOV: high-frequency oscillatory ventilation; PPHN: Persistent Pulmonary Hypertension of the Newborn; CLD: chronic lung disease; HIE: Hypoxic ischaemic encephalopathy

Figure 1: Consort diagram indicating flow of patients



MSAF: meconium stained amniotic fluid; LWN: labour ward nursery/triage nursery; TTN: transient tachypnoea of the newborn; HIE: hypoxic ischaemic encephalopathy; SAH: subarachnoid haemorrhage; ICU: intensive care unit. PNW: post natal wards.

Appendix 1: Author Guideline for the Journal of Perinatology



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Appendix 2: Ethics Approval

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr ML Ali-Dikole

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M190220

NAME: (Principal Investigator)	Dr ML Ali-Dikole
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Chris Hani Baragwanath Academic Hospital
PROJECT TITLE:	Clinical characteristics, management and outcome of neonates exposed to meconium stained amniotic fluid at Chris Hani Baragwanath Academic Hospital
DATE CONSIDERED:	2019/02/22
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Drs R Thomas and A van Kwawegen
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2019/04/04

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **February** and will therefore reports and re-certification will be due early in the month of **February** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

19.04.2019
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 3: Approved Research Protocol

University of the Witwatersrand

Faculty of Health Sciences

A review of the clinical characteristics, management and Outcomes of Neonates Exposed to Meconium Stained Amniotic Fluid at Chris Hani Baragwanath Academic Hospital

For the Degree of Master in Medicine- Paediatrics

Researcher: Dr Masida Linda Ali-Dikole

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Supervisor: Dr Reenu Thomas

Co-Supervisor: Dr Alison Van Kwawegen

TABLE OF CONTENTS

1. ABBREVIATIONS

APGAR: Activity Pulse Grimace Appearance Respiration
BPD: Bronchopulmonary dysplasia
CHBAH: Chris Hani Baragwanath Academic Hospital
CLD: Chronic Lung Disease
CTG: Cardiotocography
CMV: Conventional Mechanical Ventilation
GIT: Gastrointestinal tract
HIE: Hypoxic ischaemic encephalopathy
HFOV: High Frequency oscillator Ventilation
MAS: meconium aspiration syndrome
MSAF: Meconium stained amniotic fluid
MSL: Meconium stained liquor
NICU: Neonatal intensive care unit
NPO2: nasal prong oxygen
NCPAP: Nasal Continuous Positive Airway Pressure
PDA: Patent ductus arterious
PPHN: Persistent Pulmonary Hypertension of the Newborn

2. BACKGROUND

During the intrauterine life of a fetus, the amniotic fluid that surrounds it plays multiple roles essential to foetal well-being. [1]. It is for the foetus a complex and dynamic environment with protective and nutritive functions that changes as pregnancy progresses. [2]. This milieu at times becomes stained with meconium prior to or during labour with reported incidences of 5% in preterm gestations, 15-20% in term gestations and estimations of 23-52% described in post-term pregnancies [3-6].

Meconium is the first stool in newborns. It is commonly retained intra-intestinally until after delivery, yet, in utero passage can occur resulting in meconium stained amniotic fluid (MSAF). [7-9]. Meconium has been described as a viscous, sticky substance, of dark, olive green colour usually, made of cellular debris, amniotic fluid, mucus, water, bile acids, bile, blood, lanugo, swallowed vernix caseosa, gastro-intestinal and pancreatic secretions, interleukin-8 and phospholipase A2 [5,10-13]. It is present in the gastrointestinal tract of the fetus from approximately the tenth week of gestation and it is sterile prior to delivery as the large intestine

becomes colonized with bacteria subsequent to this.[11,13] The mechanism of its passage remains a subject of debate between mainly physiological maturation of the gastro-intestinal tract and evidence of fetal compromise [6, 10-12, 14, 15]. Other causes implicated in the passage of meconium in utero include: Maternal factors: substances ingested (eg. Misoprostol, castor oil, herbal remedies), hypertension, intra-uterine infections, pregnancy induced cholestasis; Foetal: hypoxia, cord compression resulting in peristalsis through the stimulation of the vagus nerve [3, 7, 8, 12, 14-17].

Some initiatives have been put forth to minimize adverse outcomes associated with MSAF. This includes the prevention of post term deliveries, amnioinfusion, closer monitoring of labour with lower threshold for assisted delivery or emergency caesarian section [18]. These initiatives do not all bear the same weight. Amnioinfusion is not recommended routinely; the maximum benefit has been observed in low resources settings where intrapartum monitoring is limited [8, 14, 17, 18]. The remaining initiatives have been reported to have produced reduction in the incidence of Meconium Aspiration Syndrome (MAS) in the last ten years [7, 14]. Following delivery of the newborn, the previous teaching of routine oral and/or intranasal and postnatal endotracheal suctioning of vigorous neonates is no longer recommended [8, 19]. The current practice is to observe all infants exposed to MSL for at least 24 hours and to admit those with signs of respiratory distress, ideally in a Neonatal intensive care unit (NICU) setting, with further management based on their clinical picture with anticipation of rapid deterioration in the event of disease progression [20].

The management of neonates exposed to MSAF has evolved with time guided by the growing understanding of the pathophysiology of MAS as well as asphyxia [21]. The evolution took a journey from the suctioning of all babies to suctioning no longer being a routine in neonatal resuscitation [8, 10, 19, 22]. The latest evidence has shown no benefits to sectioning vigorous newborns exposed to MSAF [10, 21, 22]. In addition, there is currently no evidence for routine suctioning of non-vigorous infants unless there is a strong suggestion of obstruction [23]. The focus has essentially shifted from clearing out meconium to helping babies breathe as early as possible to avoid complications associated with hypoxia [23, 24].

MSAF can have deleterious effect on the infant with short and long-term consequences [12]. Literature describes that not all infants exposed to MSAF develop complications, with some being more severe than others [12]. Meconium's effect on exposed tissue depends on factors

such as: concentration, duration of exposure as well as the presence of stress factors [5]. In the short term, the following are the associations of MSAF: Respiratory e.g. Meconium Aspiration Syndrome (MAS), air leaks, respiratory failure ; Neonatal Intensive Care Unit admissions with protracted stay; Cardiovascular: Persistent Pulmonary Hypertension Of The Newborn (PPHN), hypotensive shock, myocardial dysfunction, injury to the umbilical cord vessels; Infectious: neonatal sepsis; Neurological: Poor APGAR scores with increased need for resuscitation, Perinatal asphyxia, seizures, Grade 3-4 Intra-ventricular haemorrhages (IVH); GIT: feeding intolerance; Dermatological: higher rates of erythema toxicum neonatorum and physiological desquamation of the skin [4-6,8,17,25,26].

Long term complications associated with MSAF have also been reported. These include Respiratory e.g. bronchopulmonary dysplasia, reactive airway disease, decrease in lung function; Neurological e.g. cerebral palsy, autism Spectrum Disorder, Seizures, neurodevelopmental impairment [5-8,16,19,28-31]. A number of studies have also reported an increased rate of perinatal death in neonates exposed to MSAF. Shaikh et al reported mortality rates of 27.3% in their study with a much higher rate of 46.9% reported by Mesumbe et al in a different setting. [6,25,32]. The former was a cross-sectional study in a children's hospital in Pakistan and the later a study conducted in two referral centers, with likely more high risk pregnancies sent through for advanced care [25, 32].

The incidence of MAS varies across different settings where access to healthcare, level of expertise and overall resources available differ. In developed countries rates of MAS have been reported to be between 0.2% and 5% [6, 7, 9, 13, 27]. There is limited data on the incidence in developing countries, with reported rates varying from 2% - 14% [18, 32].

MAS is defined as a clinical triad of MSAF, respiratory distress with typical radiological features noted [8] It is a multifactorial disease with various pathophysiological processes [13]. These processes include airway obstruction, inactivation of surfactant and chemical pneumonitis, amongst others [33]. MAS is graded clinically based on the level of intervention required. In mild MAS, the infant requires less than 40% oxygen for less than 48 hours; in moderate MAS, the neonate requires more than 40% oxygen for more than 48 hours with no air leak syndromes and severe MAS if the infant requires assisted ventilation for more than 48 hours [8]. Complications associated with MAS include air leaks, PPHN, hypoxic ischemic encephalopathy (HIE), shock, myocardial dysfunction, chronic lung disease in term infants [19,

25]. Death is an important outcome in patients affected by MAS with mortality rates reported between 1.2 to 32% [5, 7, 11, 25, 29, 34-36].

At Chris Hani Baragwanath Academic Hospital there are a high proportion of babies born through MSAF. The prevalence, management and outcome of these babies have not been studied in the local setting and there is also a paucity of South African data. Therefore, this study aims to fill this gap in knowledge.

3. AIM OF THE STUDY

The aim of this study is to evaluate the incidence, management and outcomes of neonates born through MSAF at Chris Hani Baragwanath Academic Hospital (CHBAH) from the 1st of January to the 30th of June 2018.

4. OBJECTIVES OF THE STUDY

4.1. Primary objective

a. To determine the incidence of MAS among neonates born through MSAF.

4.2. Secondary objectives

a. Determine, in our study population, the incidence of: infants exposed to MSAF as well as their characteristics, Intrapartum hypoxia, air-leaks as well as PPHN;

b. Review the management of: infants exposed to MSAF in the delivery room; infants with MAS

c. Determine the proportion of: infants exposed to MSAF that require admission, infants with MAS that required mechanical ventilation, of infants who developed early neonatal sepsis and those who developed CLD.

d. Compare characteristics of babies who develop PPHN to those who do not, as well as that of survivors to non-survivors

e. Determine the case fatality rates and all-cause mortality of infants exposed to MSAF.

5. ETHICAL CONSIDERATIONS

Permission to conduct this study will be obtained from the Head of Department of Paediatrics as well as the Chief Executive Officer (CEO) of CHBAH. Permission will also be obtained from the Head of the Neonatal Unit for access to their REDcap database. We will apply for ethical clearance from the Human Research Ethics Committee (HREC) of The University of the Witwatersrand. Patient details will be anonymized while entering data with the use of study codes for each patient. Only the study team will have access to the patient bed letters for purposes of this research.

6. STANDARD HOSPITAL PROTOCOL FOR BABIES EXPOSED TO MSAF

The protocol at CHBAH Obstetric department for mothers in labour with MSAF involves continuous fetal monitoring via cardiotocogram and prioritizing deliveries either by means of assisted delivery or by emergency caesarian section if there is evidence of fetal distress. These deliveries are deemed high risk. Thus, one doctor from the paediatric team is requested to be present at the delivery. Resuscitation, if required, is provided at the bedside. At CHBAH recommendations from the International Liason Committee on Resuscitation (ILCOR) are followed, whereby intratracheal suctioning for non-vigorous infants is no longer routinely conducted. The aim is to establish ventilation in the first minute of life. Thus, intubation and intratracheal suctioning will only be performed if the airway is suspected to be obstructed by meconium. [23] In addition, babies born before arrival and those born in clinics around CHBAH are referred to CHBAH for assessment. After stabilization, these infants are referred to a transitional unit for observation and further management. Babies who are well will be discharged to their mothers in the post-natal ward and reassessed within 24 hours and formally discharged thereafter if well. Neonates exposed to MSAF who develop respiratory distress are managed with oxygen therapy and admitted for further management. Neonates in mild to moderate respiratory distress are managed with nasal cannula oxygen. Neonates who are in severe respiratory distress or who develop respiratory failure are intubated and ventilated in the intensive care unit (ICU). Surfactant replacement therapy is considered in patients who remain hypoxic despite being on high ventilator settings or who develop PPHN.

7. STUDY DEFINITIONS

For the purpose of this study, the following definitions will be used:

a. **Meconium Stained Amniotic Fluid**:

b. **Meconium Aspiration Syndrome**: a clinical syndrome in which a newborn delivered through MSAF develops respiratory distress requiring oxygen therapy for more than 24 hours. A chest radiograph showing characteristic radiological findings may or may not be available.

b. Ventilation for MAS: A patient meeting the definition of MAS requiring ventilation within forty-eight hours of delivery.

c. Persistent Pulmonary Hypertension of The Newborn (PPHN): persistence of high pulmonary vascular resistance resulting in right to left shunting across PDA and/or foramen ovale [36]. This may be diagnosed clinically with differential saturations between the right

arm (pre-ductal saturations) and the rest of the body (post-ductal saturations) of more than 10% or may be diagnosed on echocardiography.

d. Intrapartum hypoxia: Diagnosed if the following criteria are met: Apgar score of 5 or less at 5 minutes; arterial blood gas taken within an hour after birth showing pH of less than 7 and/or a base deficit of more than 12; evidence of encephalopathy; evidence of multi-organ dysfunction[38].

e. **Air leak syndromes**: This would include pneumothorax, pneumomediastinum, pneumopericardium and pulmonary interstitial emphysema.

f. Chronic Lung Disease: the need for supplemental oxygen for 28 days after birth with severity assessed at 36 weeks post menstrual age for babies born < or equal to 32 weeks and at 56 days or discharge (whichever comes first) for babies born > 32 weeks, based on level of support required, classified as either mild (on room air), moderate (requiring < 30% oxygen) or severe (requiring > 30% oxygen or ventilation) [37].

g. Case fatality rate: Babies who die from complications attributable to MSAF within 7 days of birth, without any other attributable cause.

8. RESEARCH METHODOLOGY

8.1. Design: This will be a retrospective descriptive study of infants exposed to MSAF delivered either at CHBAH or brought to the facility post-delivery.

8.2. Study period: 1st January 2018 to 30th June 2018.

8.3. Study population/ inclusion criteria: All neonates born through MSAF delivered at CHBAH, or brought to CHBAH post-delivery, between 1st January 2018 and 30th June 2018.

8.4. Exclusion criteria: Nil

8.5. Study setting:

The study will be conducted in the neonatal unit at CHBAH. Chris Hani Baragwanath Academic Hospital (CHBAH) lies on the outskirts of Soweto, Johannesburg, South Africa. It is a tertiary level referral hospital for all clinics in Soweto, and surrounding areas. It is also a referral hospital for surrounding primary and secondary level hospitals. The hospital conducts approximately 21,000 of the 30,000 annual births in Soweto.

8.6. Data collection:

Basic information of all newly born babies who were referred for a paediatric assessment immediately after birth is routinely recorded in the labour ward nursery and entered into a REDcap database. This information includes reason for referral, meconium exposure, maternal and neonatal demographic information, Apgar scores,

need for resuscitation, management in the nursery and outcome in the labour ward nursery. From this record we will be able to determine the proportion of babies with exposure to MSAF who were discharged to their mothers, and those who required admission. Of those who were discharged to their mothers, we will be able to extract baseline information from this database.

In addition, the neonatal unit has a REDcap database which is captured on discharge or death of a baby who was admitted to the neonatal unit. The name, hospital number and the date of birth of babies who were admitted to the neonatal unit with exposure to MSAF will be extracted from this database and using this information the neonatal bed-letters will be retrieved. These bed-letters will be reviewed and data will be collected from them for analysis. This will also then account for MSAF exposed babies who might have been discharged to their mothers but who subsequently required admission. Data collected will include demographics of the mother and the newborn, summary of antenatal history, details of resuscitation, initial blood gas, mode of respiratory support, laboratory findings, complications as well as their management and the final outcome. The total number of deliveries will be retrieved from the labour ward/ caesar theatre registers to obtain the total number of deliveries during the period under revision.

8.7. <u>Sample size</u>

Annually there are approximately 20 000 deliveries at Chris Hani Baragwanath Academic Hospital. Of these, 80% are term deliveries. Therefore, over a 6 month period we anticipate 8000 term deliveries. With the reported incidence of 15% of MSAF of all term deliveries we anticipate 1200 babies to be exposed to MSAF. The reported incidence of MAS is 14%, therefore we expect to retrieve and review approximately 168 patient files.

8.8. <u>Data analysis:</u>

Data will be captured onto Excel and analyzed using Statistica® version 12. Means and standard deviations will be used to describe continuous variables with normal distribution, and medians and ranges will be used to describe continuous variables that are not normally distributed. Frequencies and proportions will be used to describe categorical variables. To compare babies who develop PPHN to those who do not, and survivors to non-survivors, the Student t-test will be used when comparing parametric data, Mann-Whitney U test to compare non-parametric data and the Pearson Chi-square test to compare categorical variables. Differences

between the two groups will be considered to be statistically significant when the p-value is less than 0.05.

9. PROJECT OUTLINE

	Aug	Sep	Oct	Nov	Dec	Jan '19	Feb	Mar	Apr	May	Jun	July	Aug
Protocol													
Postgrad submission													
Ethics submission													
Approval													
Data collection													
Data analysis													
Write up													
Submission													

10.

10. FUNDING

The research team will privately fund the cost of stationaries and printing, with an estimated cost of R1500.

11. APPENDICES

Appendix A: Data collection sheet

Appendix A: DATA COLLECTION SHEET: RETROSPECTIVE STUDY

SECTION A: PATIENT DETAILS	
STUDY NUMBER	
DATE OF BIRTH	
DATE OF ADMISSION	
SEX	
BIRTH WEIGHT	
RACE	
GESTATIONAL AGE AT DELIVERY	
SECTION B: ANTENATAL HISTORY	
MATERNAL AGE	
PARITY AT DELIVERY	
GRAVIDITY	
IS BABY HIV EXPOSED	YES ☐ NO ☐ UNKNOWN ☐
WAS MOTHER ON ANTENATAL ART	Y ☐ N ☐ UNKNOWN ☐ N/A☐
MATERNAL COMORBIDITIES	HYPERTENSION ☐ DIABETES MELITUS ☐ INFECTIONS ☐ CARDIAC DISEASE ☐ OTHER

SECTION C: INTRAPARTUM MANAGEMENT	
SUSPICION OF CHORIOAMNIONITIS	YES ☐ NO ☐
SECTION D: BIRTH HISTORY AND DETAILS OF RESUSCITATION	
MODE OF DELIVERY	NVD ☐ C/S ☐Assisted
PLACE OF DELIVERY	INBORN ☐ OUTBORN☐
INDICATION FOR C/S	
INTRA-PARTUM ANTIBIOTIC PROPHYLAXIS	YES ☐ NO ☐ UNKNOWN ☐
1 MIN APGAR SCORE	
5 MIN APGAR SCORE 10 MIN APGAR SCORE	
TIME TO SPONTANEOUS RESPIRATION	
NEED FOR RESUSCITATION	YES ☐ NO ☐
BAG MASK VENTILATION	YES ☐ NO ☐
CPR	YES ☐ NO ☐
ADRENALINE	YES ☐ NO ☐
SUCTIONING	ORO/NASAL ENDOTRACHEAL/ Nil
INITIAL (< 1HR) BLOOD GAS FINDINGS	pH pCO2 pO2 HCO3 BE Lactate

SECTION E: RESPIRATORY SUPPORT AND MANAGEMENT	
RESPIRATORY SUPPORT	ROOM AIR ☐ NASAL PRONGS ☐ CPAP ☐ CMV ☐ HFOV ☐
IF IPPV, AGE AT INTUBATION	
INDICATION FOR INTUBATION	TYPE 1 RESPIRATORY FAILURE YES ☐ NO ☐ TYPE 2 RESPIRATORY FAILURE YES ☐ NO ☐ PPHN YES ☐ NO ☐ PPHN DIAGNOSED BY: BY ECHO YES ☐ NO ☐ BY DIFFERENTIAL SATURATIONS YES ☐ NO ☐ BOTH YES ☐ NO ☐ SEVERE RESPIRATORY DISTRESS YES ☐ NO ☐
ADJUNCTS	SURFACTANT YES ☐ NO ☐ SILDENAFIL YES ☐ NO ☐ NITRIC OXIDE YES ☐ NO ☐ INOTROPES YES ☐ NO ☐ Adrenalin/dobutamine/dopamine Hyrocortisone
LABORATORY FINDINGS	

WHITE CELL COUNT <9 X 10^9 OR > 30 X 10^9	YES ☐ NO ☐
PLATELETS < 100 X 10^9	YES ☐ NO ☐
CRP > 10mg/L	YES ☐ NO ☐
INDICATION FOR BLOOD CULTURE	EARLY NEONATAL SEPSIS ☐ LATE NEONATAL SEPSIS ☐
ORGANISM IDENTIFIED	GBS ☐ LISTERIA ☐ E.COLI ☐ PSEUDOMONAS ☐ A.BAUMANII ☐ KLEBSIELLA ☐ STAPH AUREUS ☐ CANDIDA ALBICANS ☐ CANDIDA PARAPSILOSIS ☐ ENTEROCOCCUS FECIUM ☐ ENTEROCCOCUS FAECALIS ☐ CNS ☐ OTHER ☐
SENSITIVE ORGANISM	YES ☐ NO ☐
COMPLICATIONS NOTED	
MAS	YES ☐ NO ☐

PNEUMOTHORAX	YES ☐	NO ☐	UNKNOWN ☐
PNEUMOPERICARDIUM	YES ☐	NO ☐	UNKNOWN ☐
PULMONARY INTERSTITIAL EMPHYSEMA	YES ☐	NO ☐	UNKNOWN ☐
PPHN	YES ☐	NO ☐	
ASPHYXIA	YES ☐	NO ☐	HIE1/HIE2/HIE3
CLD(OXYGEN REQUIRED FOR >28 DAYS)	YES ☐	NO ☐	UNKNOWN ☐
SEIZURES	YES ☐	NO ☐	UNKNOWN ☐
NICU ADMISSION	YES ☐	NO ☐	
MANAGEMENT OF COMPLICATIONS			
INTERCOSTAL DRAIN	YES ☐	NO ☐	
PERICARDIAL DRAINAGE	YES ☐	NO ☐	
INDUCED HYPOTHERMIA	YES ☐	NO ☐	
CXR FINDINGS consistent with MAS	YES ☐	NO ☐	
FINAL DIAGNOSIS AND SHORT TERM OUTCOME			
FINAL PRIMARY DIAGNOSIS			
OUTCOME AT DISCHARGE	SURVIVED ☐	DIED ☐	
AGE AT DISCHARGE			
LENGTH OF HOSPITAL STAY			

DISCHARGE ON HOME OXYGEN	YES ☐ NO ☐
IF DIED, CAUSE OF DEATH	ASPHYXIA YES ☐ NO ☐ PPHN YES ☐ NO ☐ SEPSIS YES ☐ NO ☐ PNEUMOTHORAX YES ☐ NO ☐

Appendix 4: Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Masida Linda Ali-Dikole (Student number: 0702627N) am a student registered for the degree of Master of Medicine in Paediatrics in the academic year 2022.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature: _____ Date: 26.07.2022

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