Statistical Analysis plan for: 'Endotheliopathy in patients with community-acquired pneumonia'

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Background and Rationale

Community-acquired pneumonia (CAP) is a frequent cause of hospital admission and mortality from pneumonia has been stable for many years.[1]

Among critically ill patients, endotheliopathy is a well-established risk factor for poor outcomes[2–5]. Syndecan-1, a proteoglycan part of the endothelial glycocalyx[6], and soluble Thrombomodulin (sTM), an endothelial cell membrane protein involved in modulating inflammation and coagulation[7–9], are both related to the risk of death and organ failure in acute respiratory failure and other types of critical illness[2,4,10,11].

Endotheliopathy, as reflected by increased plasma levels of Syndecan-1 and sTM, has been investigated and shown to be associated with poor outcomes in patients with CAP outside of the intensive care unit (ICU)[12,13], but to a much lesser extent than in critically ill patients.

We hypothesize that patients admitted with CAP suffer from endotheliopathy, but to a lesser extent than patients with established acute respiratory failure requiring mechanical ventilation. Additionally, we hypothesize that the degree of endotheliopathy at baseline is associated with oxygen requirement, respiratory deterioration and death at 30 days. To find out, we will: 1) measure Syndecan-1 and sTM in a cohort of patients with CAP and correlate the values with known risk factors for endotheliopathy; 2) compare the levels of Syndecan-1 and sTM in patients admitted with CAP with the levels of Syndecan-1 and sTM in critically ill patients requiring mechanical ventilation in the ICU; 3) correlate levels of sTM and Syndecan-1 in patients with CAP with clinical outcomes.

Methods

Study design

Surviving Pneumonia is a single-center, prospective cohort study investigating multiple aspects of patients with CAP.

Outcome Assessment

Patients are followed for collection of clinical data for entire duration of their index admission. Patient are subsequently followed for death until 2 years after their index admission.

Statistical Principles

We will consider a p-value below 0.05 significant. We will present 95% Confidence Intervals (95% CI) along with point estimates where feasible.

We will present results from both univariable and multivariable analyses where relevant.

We will not correct for multiple comparisons, as all study results will be regarded as exploratory.

Trial Population

Screening data

Patients presenting to the emergency department at North Zealand's Hospital – Hillerød with symptoms suggestive of pneumonia are continuously screened for inclusion. North Zealand's Hospital – Hillerød serves 315,000 citizens in the northern part of the Capital Region of Denmark and sees around 70,000 patients in the emergency department each year.

For the present study patients presenting from beginning of study enrolment at 1st December 2018 until 31st of December 2021 without a positive polymerase chain reaction test for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from 14 days prior to 2 days after inclusion will be considered.

We will use a cohort of critically ill patients requiring mechanical ventilation for comparison of levels of endotheliopathy biomarkers. This cohort has been described in detail elsewhere.[10]

Eligibility

Inclusion Criteria:

- Infiltrate on chest radiograph plus one or more of the following:

- Fever (temperature, ≥38.0°C)
- Hypothermia (<35.0°C),
- New cough with or without sputum production
- Pleuritic chest pain
- Dyspnea
- Altered breath sounds on auscultation.

Exclusion Criteria:

- Patients unwilling to give informed consent

Withdrawal

On patient request.

Patient Characteristics

Characteristics collected at baseline include:

- Patient demographics
- Chronic comorbidities according to Charlson Comorbidity Index
- Data on use of tobacco and alcohol
- Physiology at baseline (blood pressure, SpO2, heart frequency, mental status etc)
- Chronic medications
- Anthropometric measurements (height, weight etc)

Characteristics collected during index hospital admission include:

- Medications received
- Physiologic parameters once daily
- Microbiological investigations
- Complications of pneumonia (abscess, pleural effusion, acute respiratory failure, ICU admission etc)

Analysis

Statistical programming software

All analyses will be performed with R, version 4.1.1[14].

Outcome definitions

The primary outcome is the need for oxygen supplement during the first five days of the index admission. Status of oxygen therapy is obtained from the early warning score in the electronic heath record (EHR). We will consider patients discharged from hospital to be without a need for supplemental oxygen therapy for the days discharged from hospital.

Secondary outcomes are the need for mechanical ventilation (invasive or non-invasive positive pressure ventilation) during index admission, admission to intensive care unit during index admission, SpO2/FiO2-ratio during the first five days of the index admission, and 30-day all-cause mortality.

Variables

We will measure Syndecan-1 and sTM in plasma from blood samples obtained at baseline. We will use commercially available enzyme-linked immunosorbent assays according to the manufacturers' instructions.[15,16] A total of 175 microliter of plasma is needed for the two analyses.

Based on the directed acyclic graphs[17] of the expected causal relationships found in CAP between different risk factors and endotheliopathy (Figure 1 to 6) we will correct for the expected confounders and evaluate the association between endotheliopathy and age, sex, arteriosclerosis, diabetes, chronic kidney disease and pneumonia severity respectively.

Comparing levels of Syndecan-1 and sTM between the patients with CAP and the cohort of critically ill patients requiring mechanical ventilation, we will correct for the variables that are significantly associated with endotheliopathy among the patients with CAP.

Based on the directed acyclic graph[17] of the expected causal relationship between endotheliopathy and the need for supplemental oxygen therapy (Figure 7), we will correct for the following confounders in the analysis of the primary outcome: age, arteriosclerosis, chronic kidney disease, diabetes and sepsis at baseline.

One author (MSL) will adjudicate the presence or absence of arteriosclerosis based on information (e.g. history of prior myocardial infarction or prior stroke) entered in the case report form (CRF).

We define diabetes as a known diagnosis of type I or II Diabetes prior to the index admission.

We define chronic kidney disease as median eGFR < 45 ml/min/1.73 m^2 based on all creatinine measurements from 180 to 8 days prior to study inclusion. We will obtain the eGFR values from the EHR.

We define sepsis in accordance with the "Third international consensus definition for sepsis" as suspected or confirmed infection plus one or more of: altered mentation, a systolic blood pressure =< 100 mmHg, a respiratory rate >= 22/minute.[19]

In the analysis of the secondary outcome, we will correct for the CURB-65 score at admission.[20]

Analysis methods

To investigate the associations between the risk factors for endotheliopathy and endotheliopathy at baseline, we will perform multiple linear regression controlling for the appropriate confounders according to the DAG.

To compare the levels of endotheliopathy biomarkers between patients admitted with CAP and the cohort of critically ill patients requiring mechanical ventilation, we will perform multivariable linear regression controlling for the appropriate confounders.

We will analyse associations between endotheliopathy and the need for supplemental oxygen therapy during the first five days of index admission using multivariable logistic regression.

We will analyse the associations between endotheliopathy and the SpO2/FiO2-ratio during the first three days using mixed effects linear regression. For this analysis, we will consider as the primary effect measure an interaction test between the associations of time and the baseline level of Syndecan-1 and sTM with the daily SpO2/FiO2-value.

We will analyse all other secondary outcomes using multivariable Cox-regression. Regarding the need for mechanical ventilation and admission to the intensive care unit during the index admission, we will present the cause-specific hazard ratio together with that of the competing risk of death before the occurrence of the considered outcome.

We will examine all models for standard measures of model fit and assumptions and we will transform the included variables as appropriately.

Missing data

For the SpO2-FiO2-ratio in the first three days of index admission, we suspect that observations will be subject to informative missingness. That is, we suspect that patients with high values of SpO2-FiO2-ratio will be discharged and therefore be missing from the dataset, and we suspect that patients with low values of SpO2/FiO2-ratio will die and therefore be missing from the dataset. The mixed effects linear model is relatively robust against missing data, but as a sensitivity analysis we will impute[21] all missing SpO2-FiO2-ratios and rerun the models.

For all other variables, missing data will be handled as follows: if less than 2,5% of an outcome or variable is missing, missing data is handled by case-wise deletion; if more than 2,5% is missing, multiple imputation will be performed using chained equations with predictive mean matching and logistic regression for numerical and categorical variables respectively.[21] All variables and outcomes included in the models will be used to inform the imputations. 10 datasets will be imputed.

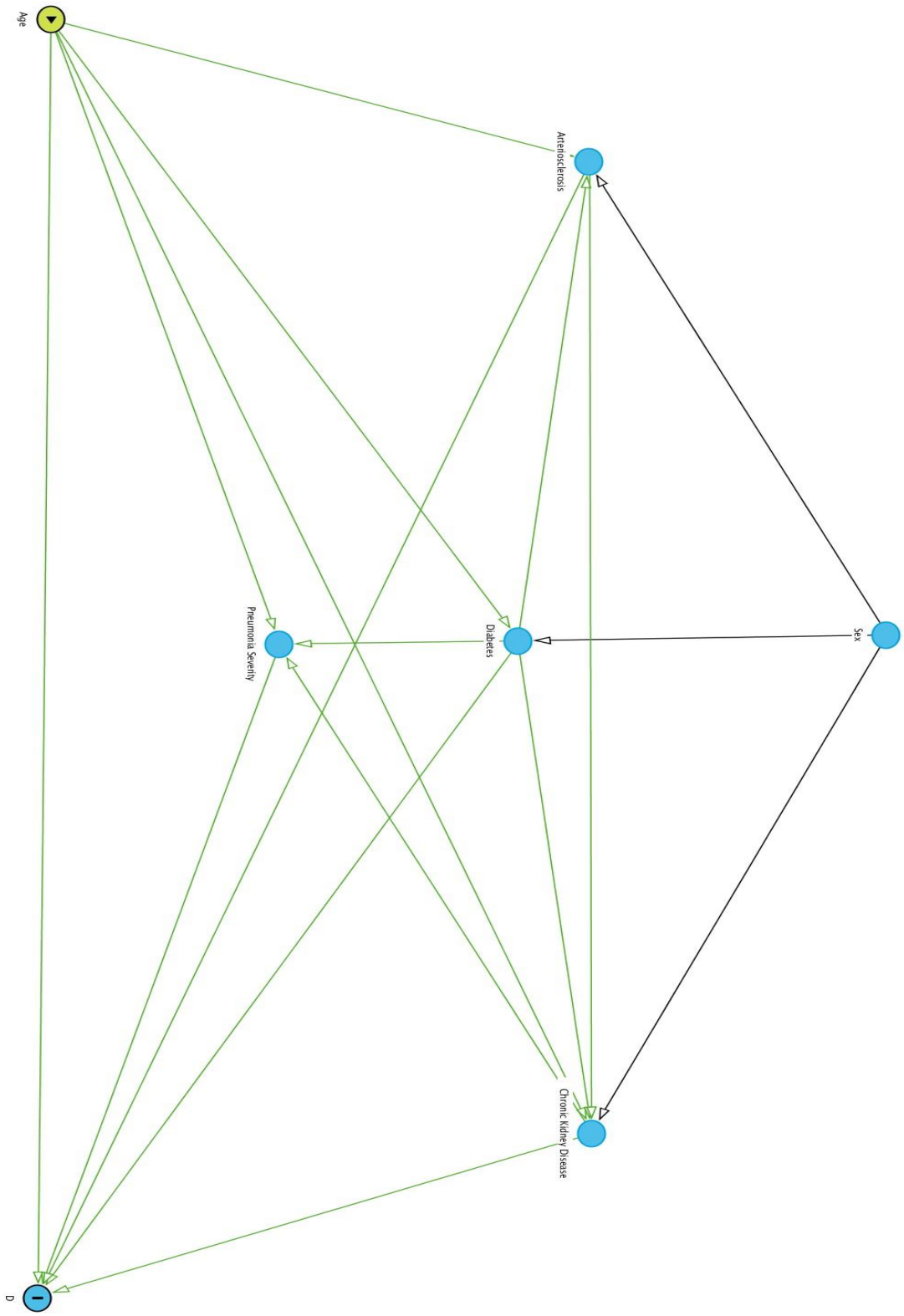

Figure 1: Directed acyclic graph of the expected causal relationsships between age and endotheliopathy.

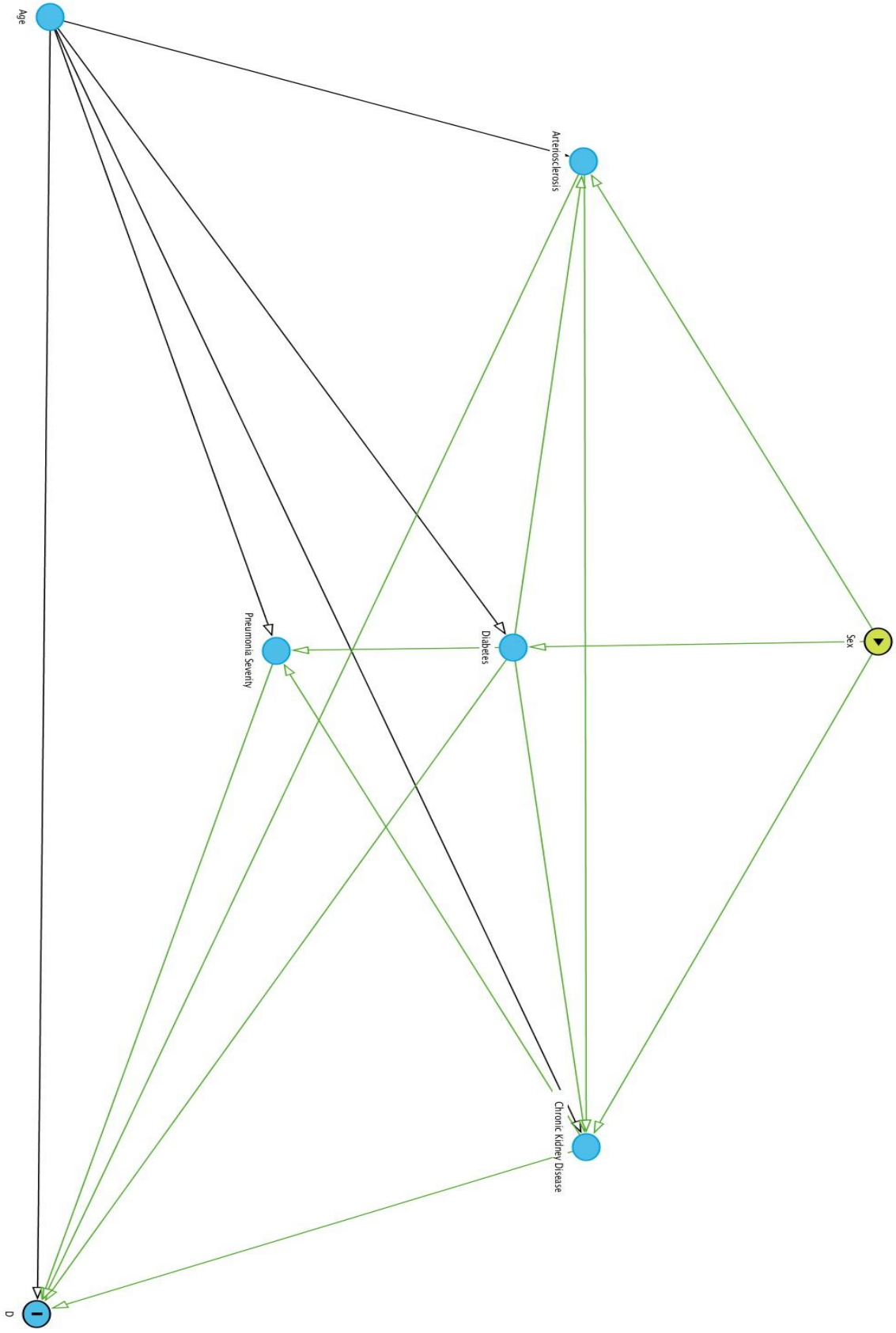

Figure 2: Directed Acyclic Graph of the expected causal relationsships between sex and endotheliopathy

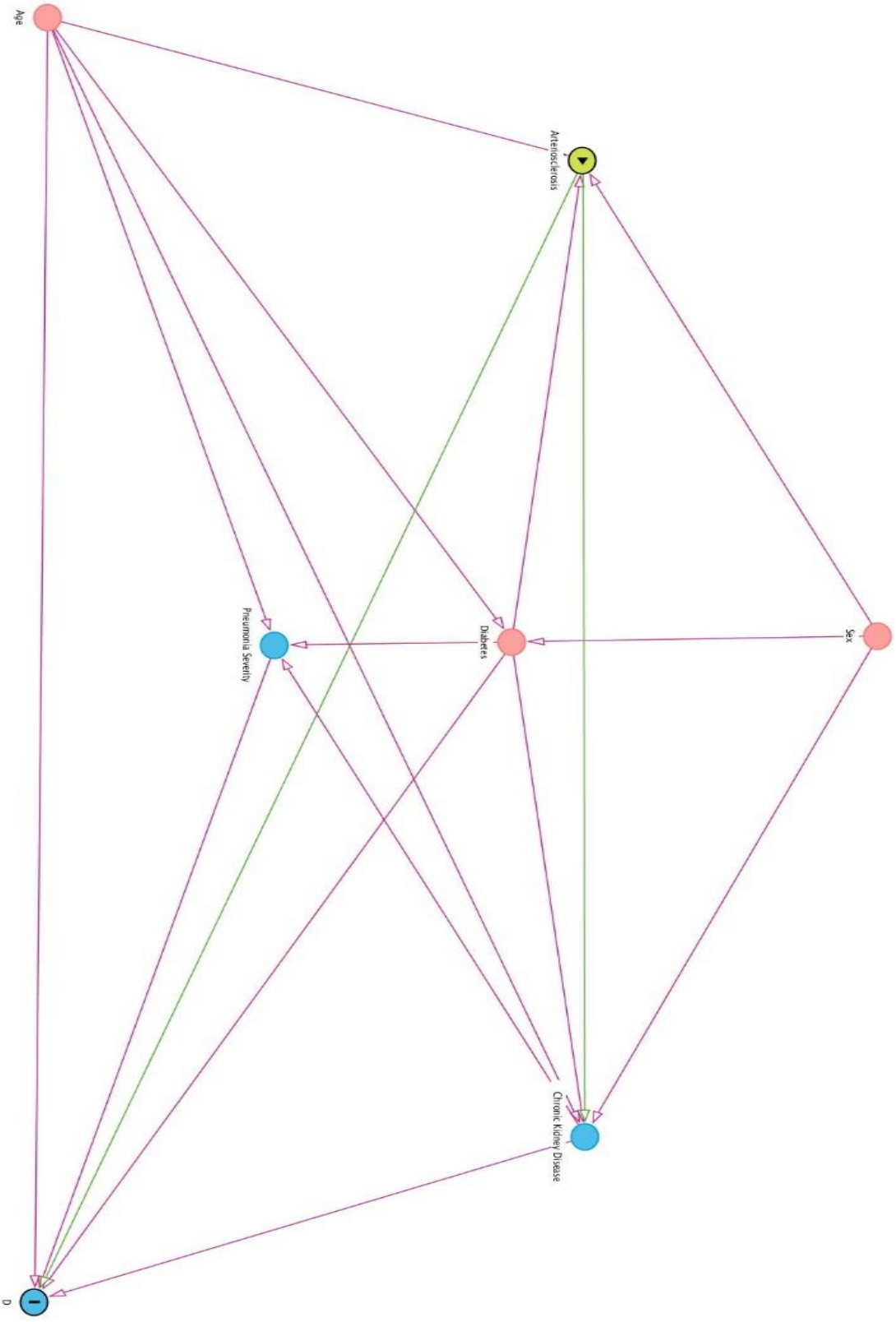

Figure 3: Directed Acyclic Graph of the expected causal relationsships between arteriosclerosis and endotheliopathy

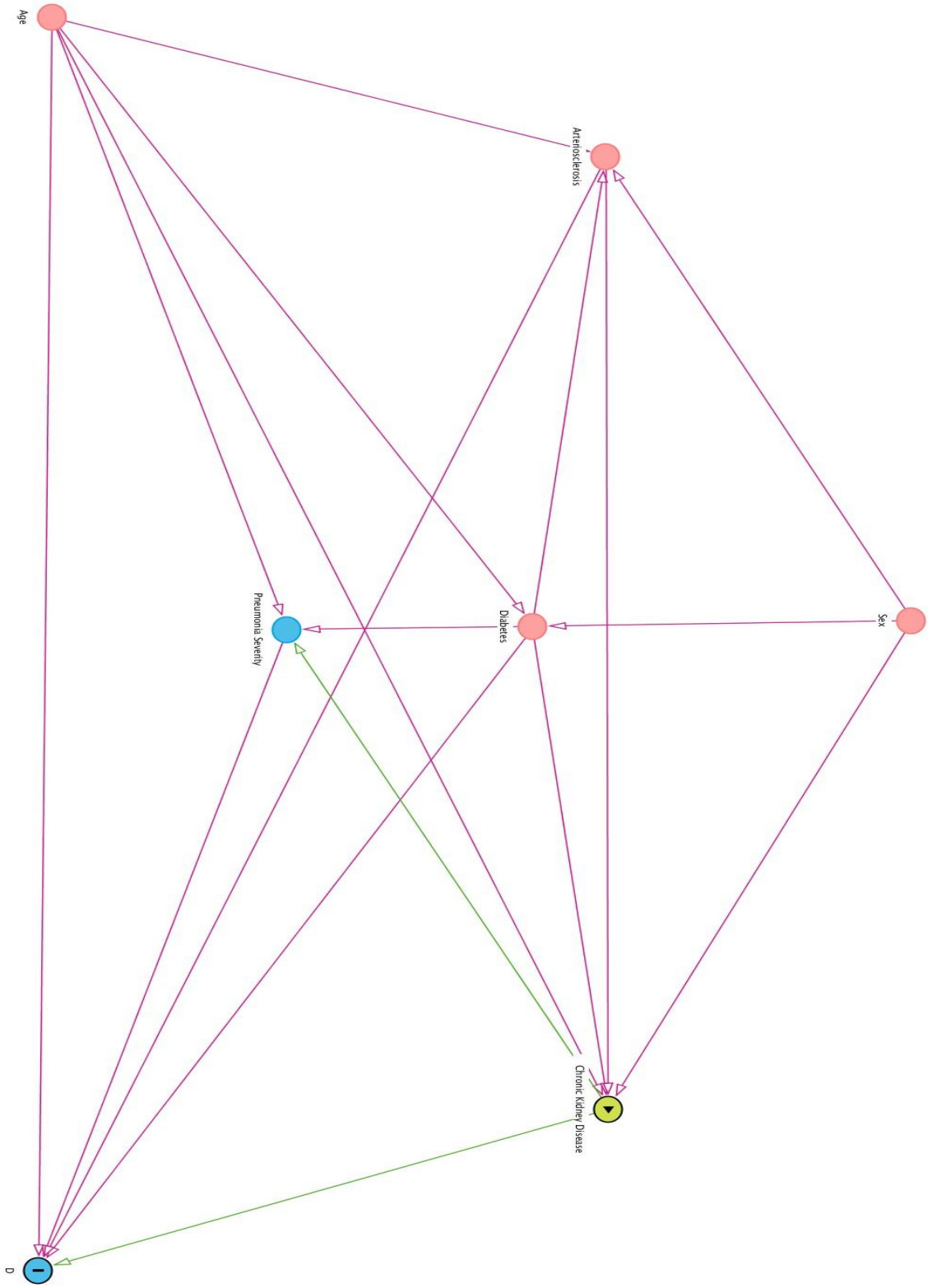

Figure 4: Directed Acyclic Graph of the expected causal relationsships between chronic kidney disease and endotheliopathy

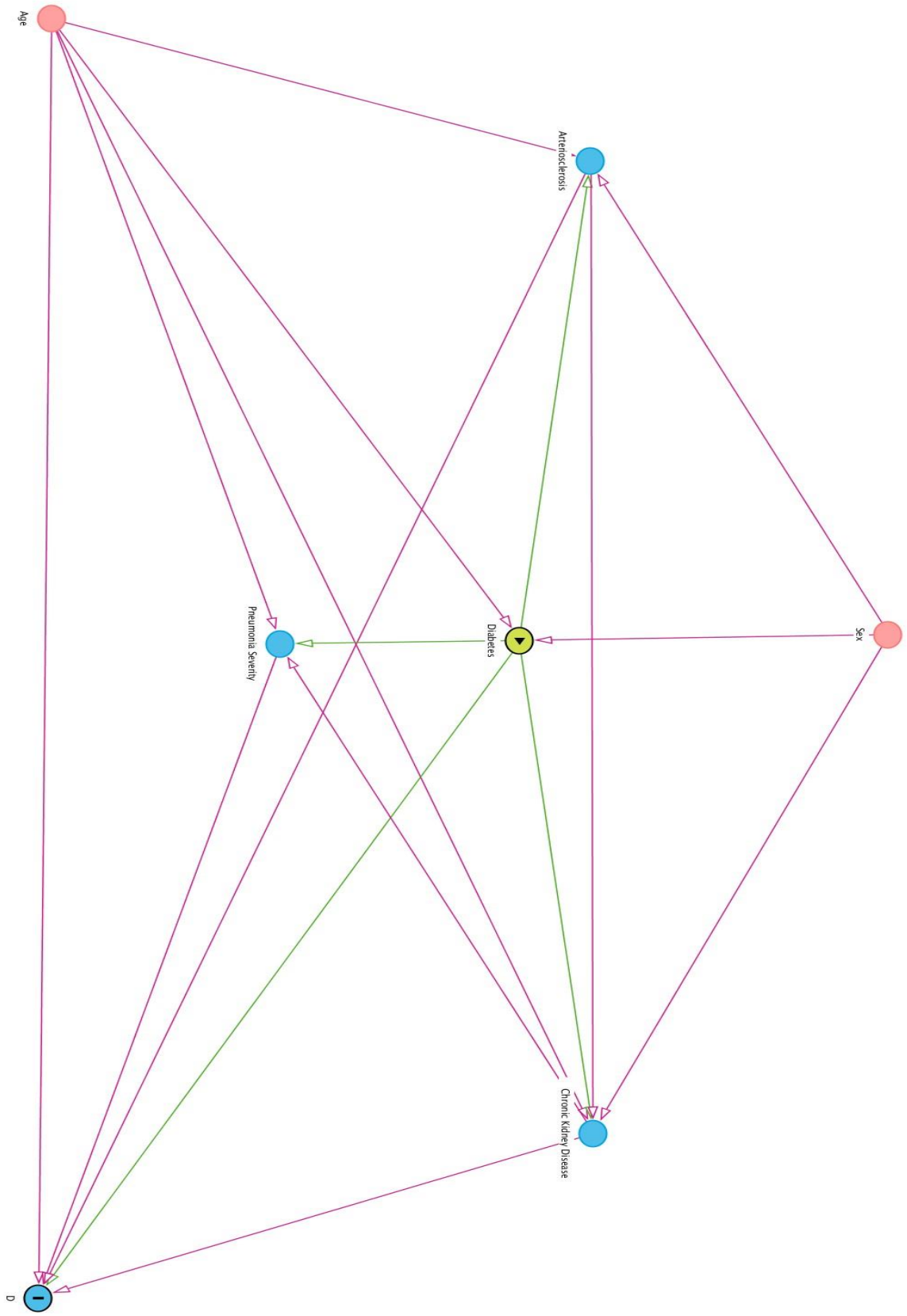

Figure 5: Directed Acyclic Graph of the expected causal relationsship between diabetes and endotheliopathy

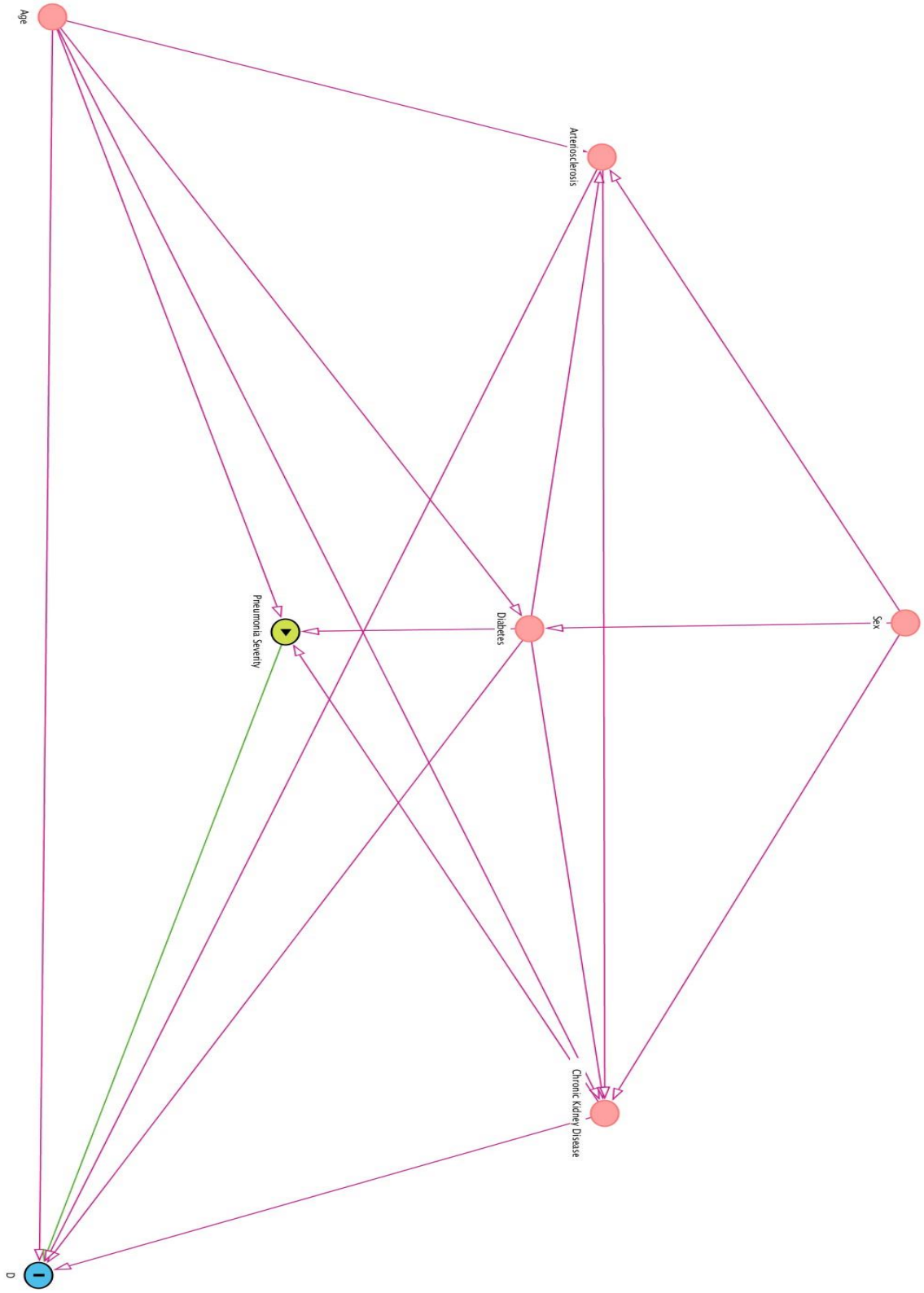

Figure 6: Directed Acyclic Graph of the expected causal relationsship between severity of pneumonia and endotheliopathy

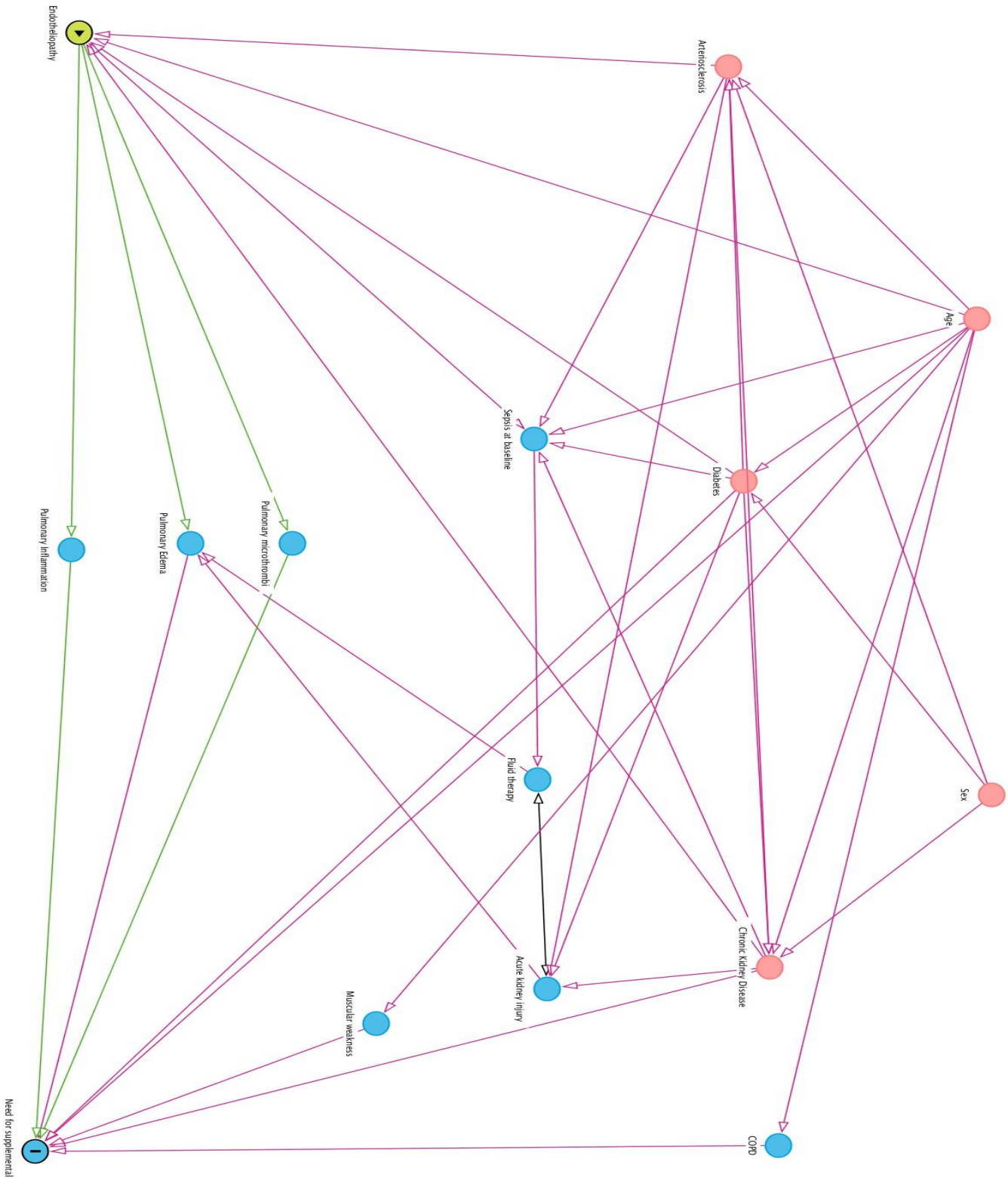

Figure 7: Directed acyclic graph of the expected causal relationsships between endotheliopathy and impaired gas exchange

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