Prognostic Factors for Evolution of Non Alcoholic Fatty Liver Disease Patients Utilizing Poisson Regression and Continuous Time Markov Chains

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Abstract

In the present paper, the deleterious effects of obesity, type 2diabetes and insulin resistance, systolic and diastolic hypertension on the rate of progression of fibrosis in non-alcoholic fatty liver disease (NAFLD) patients are illustrated using a new approach utilizing the Poisson regression to model the transition rate matrix. The observed counts in the transition counts matrix are used as response variables and the covariates are the risk factors for fatty liver. Then the estimated counts from running the Poisson regression are used to estimate the transition rates using the continuous time Markov chains (CTMC) followed by exponentiation of the estimated rate matrix to obtain the transition probability matrix at specific time points. Using a hypothetical data of 150 participants followed up every year for a total of 28 years recording their demographic characteristics and their timeline of follow up are demonstrated. The findings revealed that insulin resistance expressed by MOMA-IR 2 has the most deleterious effects among other factors for increasing the rate of forward progression of patients from state 1 to state 2 as well as from state 2 to state 3 and from state 3 to state 4. The higher the level of HOMA-IR is, the more rapid the rate of progression is.

Key words: Continuous time Markov chains, Life expectancy, Maximum Likelihood estimation, Mean Sojourn Time, Non-Alcoholic Fatty Liver Disease, Panel Data.

Introduction

Continuous time Markov chains (CTMC) are valuable and of great potentiality mathematical and statistical tools to be used for evaluation of disease progression over time. CTMCs are a subtype of multistate models to be utilized to study this progression in NAFLD patients, with its characteristic phenotypes NAFLD and NASH, hand in hand with the presence of associated fibrosis and its stages. The prevalence of NAFLD is quickly growing worldwide, and matches the epidemics of obesity and type 2diabetes. Metabolic syndrome is a well-known risk factor which requires the presence of abdominal obesity distinguished by waist circumference >94 cm for males and >80 cm for females in eastern countries while it is >120 cm for males and >88 cm for females in the western countries, plus 2 or more of the following: blood glucose ≥100 mg/dL or drug treating diabetes, arterial blood pressure ≥ 130/85 mmgh or drug treating hypertension, triglyceride levels ≥150mg/dL or drug treating increased levels in blood or high density lipoprotein (HDL) levels <40 mg/dL for males and <50 mg/dL for females or drug treating this condition.

NAFLD can be modeled using the simplest form for health, disease, and death model, with one state for susceptible individuals with risk factors, such as: type 2 diabetes, dyslipidemia and hypertension, the other state is the NAFLD phenotypes, and two competing states for death: one for liver-related mortality as a complication of NAFLD, and the other death state is death causes unrelated to liver disease (Younossi et al., 2016). This is shown in figure 1:

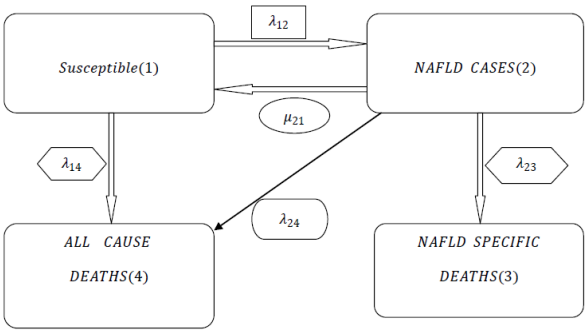


Figure 1: General Model Structure

In addition, NAFLD is modeled in more elaborative expanded form, which includes nine states: the first eight states are the states of disease progression as time elapses; while, the ninth state is the death state (Younossi et al., 2016) , as illustrated in figure 2:

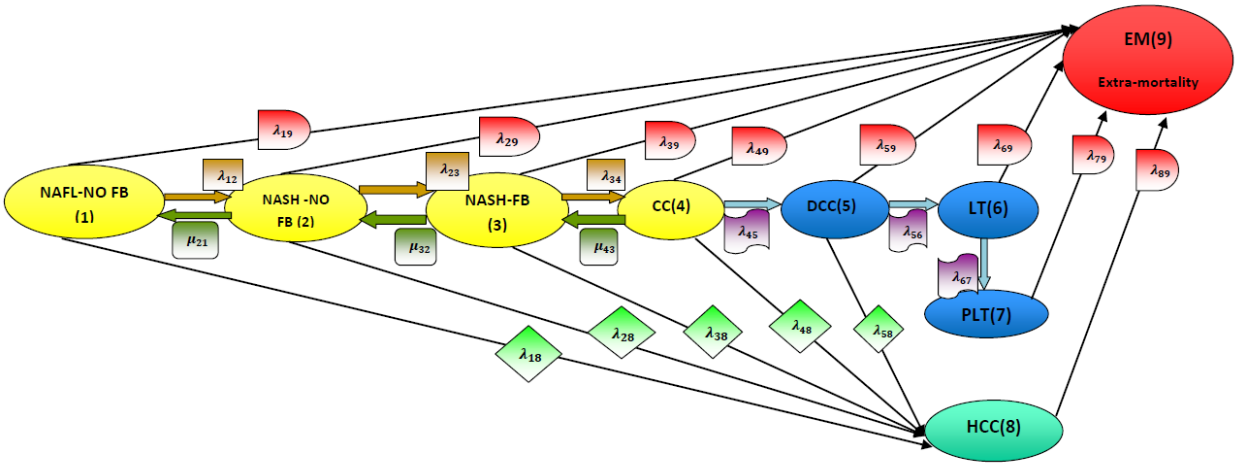


Figure 2: disease model structure:
NAFLD-NO FB = nonalcoholic fatty liver disease with no fibrosis (stage 1). NASH-NO FB = nonalcoholic steato-hepatitis with no fibrosis (stage 2). NASH-FB = nonalcoholic steato-hepatitis with fibrosis (stage 3). CC= compensated cirrhosis (stage 4). DCC= de-compensated cirrhosis (stage 5). LT= liver transplant(stage 6). PLT =post liver transplant (stage 7). HCC =hepato-cellular carcinoma (stage 8). EM= extra-mortality (stage 9).

Moreover, a subset of the states that explicitly illustrates the phases of fibrosis process, which develops early in disease evolution cycle if the risk factors are not treated or eliminated, is modeled with CTMC to demonstrate: how covariates incorporated in a log-linear model can relate these predictors to transition rates among states, as illustrated in figure 3 (Younossi et al. 2020),(Singh et al. 2015). The presence of fibrosis is considered an ominous predictor for disease progression. This subset is a subset of states from the expanded model especially early phases or stages where reversibility of conditions in each stage can be achieved if properly treated and controlled so as to prevent reaching the irreversible damaged state which is liver cirrhosis or F4.

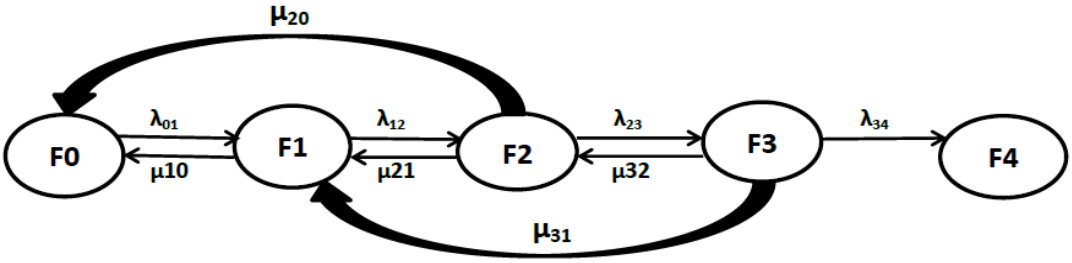


Figure 3: NAFLD with the evolving fibrosis stages.
F0= no fibrosis (stage 0) whether hepatic steatosis is present or not . NASH-FB-1 =nonalcoholic steatohepatitis with mild fibrosis (stage 1). NASH -FB-2 = NASH with moderate fibrosis (stage 2). NASH -FB-3 = NASH with advanced or severe fibrosis (stage 3). CC= compensated cirrhosis (stage 4) which is the more severe or advanced form of fibrosis.

Singh et al. 2015 conducted a meta-analysis to evaluate the rate of fibrosis progression and thus searched multiple databases through a thoroughly systematic manner associated with author contact and found 11 cohort studies on NAFLD adult patients having at least one year apart paired liver biopsy specimens, from which they calculated a pooled-weighted annual fibrosis progression rate (number of stages changed between the 2 biopsy samples) with 95% confidence interval (CIs), and characterized the clinical risk factors accompanying this progression. They identified 411 patients with biopsy-proven NAFLD (150 with NAFL and 261 with NASH) included in those studies. Initially, the distribution of fibrosis for stages 0,1,2,3 and 4 was 35.8%, 32.5%, 16.7 %, 9.3% and 5.7% respectively, and over 2145.5 person-years of follow-up evaluation, 33.6% had fibrosis progression, 43.1% had stable fibrosis, and 22.3% had an improvement in fibrosis stage. The annual fibrosis progression rate in patients with NAFL who had stage 0 fibrosis at baseline was .07 stages (95% CI, 0.02-0.11 stages), compared with 0.14 stages in patients with NASH (95% CI, 0.07-0.21 stages). These findings correspond to 1 stage of progression over 14.3 years for patients with NAFL (95% CI, 9.1-50.0 y) and 7.1 years for patients with NASH (95% CI, 4.8-14.3 y).

(Kalbfleisch & Lawless, 1985) related the instantaneous rate of transitions from state i to state j to covariates, by regression modeling of the Q transition rate matrix using log-linear model for the Markov rates.

In the present study, Poisson regression is used to model the rates among states. The counts of each transition can be modeled as a function of some explanatory variables reflecting the characteristics of the patients. This can be accomplished by using Poisson regression model or log-linear model. The Poisson regression model specifies that each response y_i is drawn from a Poisson population with parameter λ_i , which is related to the regressors or the covariates. The primary equation of the model is

$$P(Y = y_i|x_i) = \frac{e^{-\lambda_i}\lambda_i^{y_i}}{y_i!}$$

The most common formulation for the λ_i is the log-linear model:

$$\ln \lambda_i = x_i'B$$

And the expected number of events per period is given by:

$$E[y_i|x_i] = var[y_i|x_i] = \lambda_i = e^{x_i'B}$$

The observed counts in the transition counts matrix is used as response variables and the covariates are the risk factors for fatty liver. Then the estimated counts obtained from the Poisson regression model are used to estimate the rates using the CTMC, as the initially observed transition rates approximately equal the estimated transition rates among states, as illustrated by the author in previous 2 papers, followed by exponentiation of the estimated rate matrix. To expound this procedure a hypothetical example is used , and it is in the form of a study conducted on 150 participants over 28 years to follow the progression of the NAFLD from F0 to F4.

An illustrative hypothetical example is demonstrated in this paper high-lightening the verification of assumption of Poisson regression, discussion of the results, and model diagnostics. Supplementary materials are complementary to this paper and contain more tables, figures and discussions.

1. Study Design

One hundred fifty participants were followed up every year for 28 years, and at each visit the characteristics of the participants were recorded like sex(0=female,1=male),age, BMI, LDL-chol, HOMA2_IR, systolic blood pressure as well as the diastolic pressure as shown in the table (1) (see supplementary materails).

1.1 statistical summaries of the participants:

For each participant the recorded value in the table is the mean of the follow up measurements. Fitting the Poisson regression and the estimated counts for each transition were calculated using Stata 14. A summary statistics for the patients' characteristics is shown in table (2) (see supplementary materails). The output results of summary statistics in Stata is illustrated below:

```
. sum  age LDL_chol HOMA2_IR BMI sysBloodPr diastBloodPressure
```

Variable	Obs	Mean	Std. Dev.	Min	Max
age	150	40.20667	4.928069	27	53
LDL_chol	150	94.80755	15.40653	59.88626	133.1309
HOMA2_IR	150	2.278219	.709836	.4866602	4.361196
BMI	150	28.28346	2.990533	20.30083	35.16374
sysBloodPr	150	149.7291	10.43408	123.4009	175.7543
diastBlood~e	150	94.25166	11.38758	69.99635	124.0379

```
. tab gender
```

gender	Freq.	Percent	Cum.
0	69	46.00	46.00
1	81	54.00	100.00
Total	150	100.00	

The participants were categorized according to these demographic characteristics as shown in table (3) (see supplementary materails), while in table (4) summary of the categorical groups according to the participants' characteristics like: age category BMI category, LDL-chol category, systolic and diastolic blood pressure category (see supplementary materails)

There are high correlations between the continuous predictor variables as shown in table (5) (see supplementary materails). The output results of correlation in Stata are illustrated below:

```
. corr age LDL_chol HOMA2_IR BMI sysBloodPr diastBloodPressure
(obs=150)
```

	age	LDL_chol	HOMA2_IR	BMI	sysBlo~r	diastB~e
age	1.0000					
LDL_chol	0.9919	1.0000				
HOMA2_IR	0.9941	0.9947	1.0000			
BMI	0.9938	0.9948	0.9960	1.0000		
sysBloodPr	0.9958	0.9953	0.9958	0.9962	1.0000	
diastBlood~e	0.9915	0.9951	0.9962	0.9945	0.9949	1.0000

In table (6) (see supplementary materials) the transition counts accomplished by each participant in these 28 years are illustrated. Summary of observed transition counts among the states in these 28 years is clarified in table (7). The timeline for each participant is shown in table (8) (see supplementary materials) with first column is t=0 and the last column is t=28 and in each of these column(year) the state of the patient was recorded. The observed transition counts are illustrated in table (9).

Table (7): summary of observed transition counts between the states

Counts	**Transition 0→1**	**Transition 1→2**	**Transition 2→3**	**Transition 3→4**	**Transition 1→0**	**Transition 2→1**	**Transition 3→2**	**Transition 2→0**	**Transition 3→1**
0	63	96	121	128	121	127	130	138	139
1	58	43	23	22	24	17	17	11	9
2	25	9	4		3	5	3	1	2
3	4	2	2		2	1			
Total	150	150	150	150	150	150	150	150	150

Table (9): Observed transitions counts of the patients over the 28 years

	State 0	State1	State2	State3	State4	total
State0	1909	120	15	6	0	2050
State1	36	1116	67	28	0	1247
State2	13	30	703	37	0	783
State3	11	14	23	50	22	120
State4	0	0	0	0	0	0
						4200

Initial observed rates are:

$$\lambda_{01} = \frac{120}{2050} = .059\ , \lambda_{12} = \frac{67}{1247} = .0537\ ,\ \lambda_{23} = \frac{37}{783} = .047\ \ , \lambda_{34} = \frac{22}{120} = .183$$

$$\mu_{10} = \frac{36}{1247} = .0288\ , \mu_{21} = \frac{30}{783} = .0383\ , \mu_{32} = \frac{23}{120} = .191\ , \mu_{20} = \frac{13}{783} = .016\ , \mu_{31} = \frac{14}{120} = .116$$

Using CTMC, the estimated rates approximately equal the initially observed rates, as illustrated by the author Iman Attia in previous 2 papers utilizing the simplest small model and the expanded model, where no covariates were included in the analysis.

1.2 Verification of Assumption:

The distribution of the transition counts among the states is Poisson as illustrated by the following histogram for each transition count and mostly the index of dispersion for counts among states is one. The output results of the histogram of each transition count and the associated dispersion index for this count are shown below:

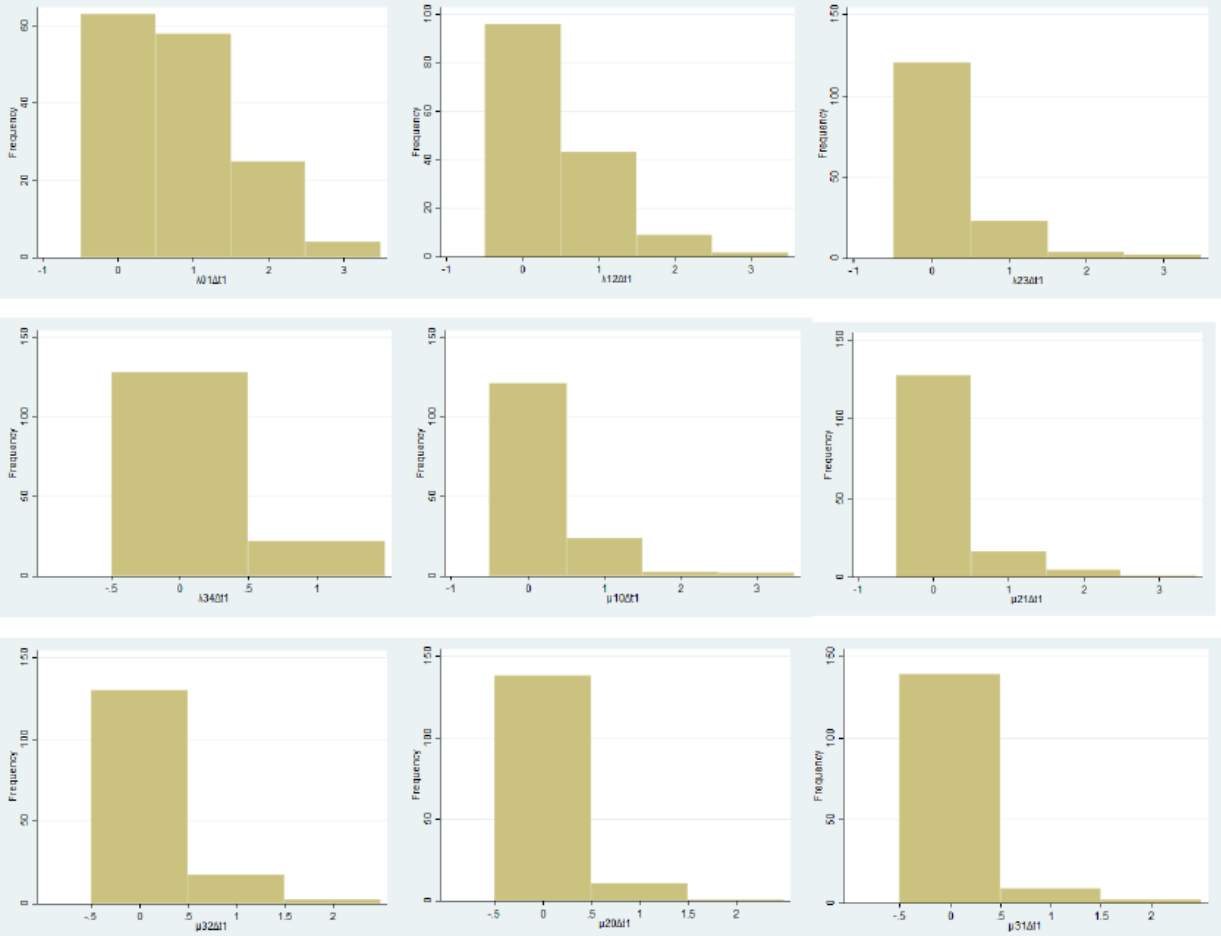


Figure 4: the histograms for the counts of transitions among different states.

```
. sum λ01Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
λ01Δt1	150	.8	.8109982	0	3

```
. di r(sd)^2/r(mean)
.82214765

. sum λ12Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
λ12Δt1	150	.4466667	.6709371	0	3

```
. di r(sd)^2/r(mean)
1.0078133

. sum λ23Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
λ23Δt1	150	.2466667	.5668311	0	3

```
. di r(sd)^2/r(mean)
1.3025576

. sum λ34Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
λ34Δt1	150	.1466667	.3549585	0	1

```
. di r(sd)^2/r(mean)
.8590604

. sum μ10Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
μ10Δt1	150	.24	.5517513	0	3

```
. di r(sd)^2/r(mean)
1.2684564

. sum μ21Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
μ21Δt1	150	.2	.5181278	0	3

```
. di r(sd)^2/r(mean)
1.3422819

. sum μ32Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
μ32Δt1	150	.1533333	.4134755	0	2

```
. di r(sd)^2/r(mean)
1.1149694

. sum μ20Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
μ20Δt1	150	.0866667	.3051387	0	2

```
. di r(sd)^2/r(mean)
1.0743418


. sum μ31Δt1
```

Variable	Obs	Mean	Std. Dev.	Min	Max
μ31Δt1	150	.0866667	.3263931	0	2

```
. di r(sd)^2/r(mean)
1.2292204
```

The above are the output results of the associated dispersion indices for these counts, both the histograms and the dispersion indices highly suggest that the distribution of the transitions counts among states are Poisson distribution since almost the variance equals the mean. The predictors are continuous variables and the observations are independent from each other.

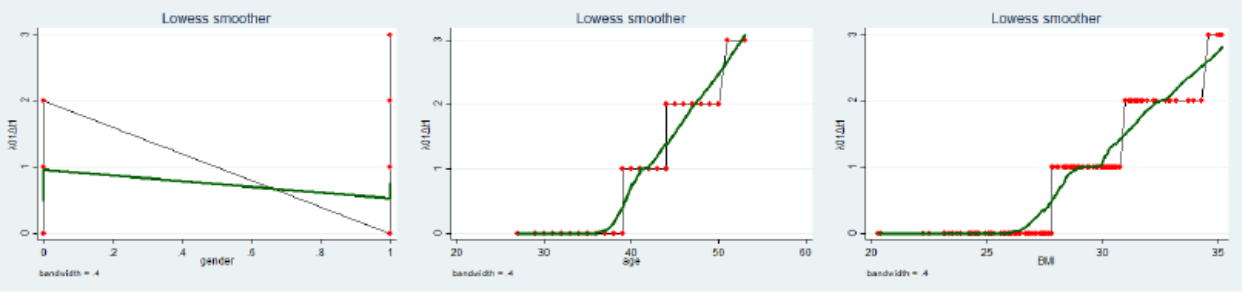
1.3 Functional Form of the Variables:

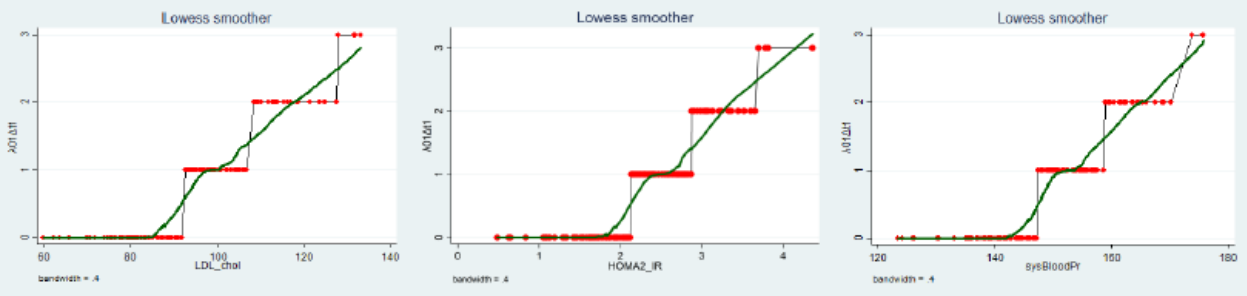
Lowess smoother illustrates that the relationships between each of the response transition count and each variable is not strictly linear, but it is curvilinear relationship, with initial part of this relation being nearly horizontal and it starts to curve upwards at some predictor point located inside the second category of each predictor. And the followings are the output results of Lowess smoother commands and associated figures demonstrating the relationship between the transition counts for the movement from stage 0 to stage 1 with each of the predictor variable, the other transition counts are illustrated in the (supplementary materials):

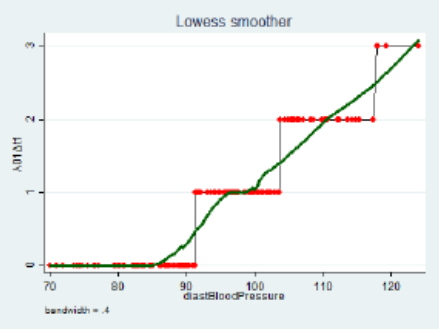
```
. lowess λ01Δt1 gender, bwidth(0.4) recast(connected) mcolor(red) msize(medlarge) lwidth(medthin) lineopts(lcolor(dkgreen) lwidth(thick)
>  lpattern(solid) connect(direct))

.
. lowess λ01Δt1 age, bwidth(0.4) recast(connected) mcolor(red) msize(medlarge) lwidth(medthin) lineopts(lcolor(dkgreen) lwidth(thick) lp
> attern(solid) connect(direct))

.
. lowess λ01Δt1 LDL_chol, bwidth(0.4) recast(connected) mcolor(red) msize(medlarge) lwidth(medthin) lineopts(lcolor(dkgreen) lwidth(thic
> k) lpattern(solid) connect(direct))

.
. lowess λ01Δt1 BMI, bwidth(0.4) recast(connected) mcolor(red) msize(medlarge) lwidth(medthin) lineopts(lcolor(dkgreen) lwidth(thick) lp
> attern(solid) connect(direct))

.
. lowess λ01Δt1 HOMA2_IR, bwidth(0.4) recast(connected) mcolor(red) msize(medlarge) lwidth(medthin) lineopts(lcolor(dkgreen) lwidth(thic
> k) lpattern(solid) connect(direct))

.
. lowess λ01Δt1 sysBloodPr, bwidth(0.4) recast(connected) mcolor(red) msize(medlarge) lwidth(medthin) lineopts(lcolor(dkgreen) lwidth(th
> ick) lpattern(solid) connect(direct))

.
. lowess λ01Δt1 diastBloodPressure, bwidth(0.4) recast(connected) mcolor(red) msize(medlarge) lwidth(medthin) lineopts(lcolor(dkgreen) l
> width(thick) lpattern(solid) connect(direct))
```

Relationship between number of transitions from state 0 to state 1 starts to bends up where each of the six predictors are located inside the second category; where age is approximately ≥37, BMI is approximately ≥ 26, LDL-chol is approximately ≥ 85 mg/dL, HOMA-IR is approximately ≥1.7, systolic blood pressure is approximately 142 mmHg, and diastolic blood pressure is approximately ≥ 85 mmHg. All these values are located in the second category as illustrated in the following output results of Stata below, the same procedures are done for the other transition counts which are illustrated in the (supplementary materials):

Figure(5) illustrates the relationship between transition counts from state 0 to state 1 and each predictors as explained by lowess smoother.







This can give good orientation to the functional form of the variables to be used in the regression model and avoid the misspecification resulting from mal-functional form of the predictors.Thus, the restricted cubic splines are used for the predictors with 5 knots using Harrell approach which is the default procedure utilized by Stata 14 software. The locations of knots and the correlations between the transformed variables are illustrated on the output results in Stata as shown below:

```
. mkspline HOMAsp = HOMA2_IR, cubic displayknots
```

	knot1	knot2	knot3	knot4	knot5
HOMA2_IR	1.0879	1.802248	2.263381	2.752956	3.480719

```
.
. mkspline LDLsp = LDL_chol, cubic displayknots
```

	knot1	knot2	knot3	knot4	knot5
LDL_chol	71.22266	83.70328	94.62621	104.4809	124.1413

```
.
. mkspline sysPS = sysBloodPr, cubic displayknots
```

	knot1	knot2	knot3	knot4	knot5
sysBloodPr	133.0965	143.8759	149.4107	155.5788	168.0359

```
.
. mkspline DiasPS = diastBloodPressure, cubic displayknots
```

	knot1	knot2	knot3	knot4	knot5
diastBlood~e	74.44541	87.43943	94.06548	101.1098	114.4986

For each variable, four different functions have been created and these are the correlations between the relevant transformed variables used in the Poisson regression as illustrated in the stata output results below:

```
. corr HOMAsp1 HOMAsp2 LDLsp2 sysPS2 DiasPS2
(obs=150)
```

	HOMAsp1	HOMAsp2	LDLsp2	sysPS2	DiasPS2
HOMAsp1	1.0000				
HOMAsp2	0.8869	1.0000			
LDLsp2	0.8572	0.9893	1.0000		
sysPS2	0.8674	0.9908	0.9959	1.0000	
DiasPS2	0.8854	0.9950	0.9944	0.9929	1.0000

The Poisson regression was applied using the observed counts of the transition counts matrix as response variable, and the following results are obtained as discussed below in the next section.

2. Results and Discussion:

In the next discussion, the results of running Poisson regression to obtain the following estimated counts are demonstrated. Running Poisson regression on these transformed variables gives the estimated counts shown in table (10):

Table 10: the estimated counts for each transition

Counts	Transition 0→1	Transition 1→2	Transition 2→3	Transition 3→4	Transition 1→0	Transition 2→1	Transition 3→2	Transition 2→0	Transition 3→1
0	75	102	125	133	126	132	135	140	140
1	34	35	18	14	15	12	12	8	7
2	37	11	4	3	7	4	2	2	3
3	4	1	3	0	1	2	1	0	0
4	0	1	0	0	1	0	0	0	0
Total	150	150	150	150	150	150	150	150	150

In the following output results of running Poisson regression in stata, the estimated counts of transition from state 0 to state 1 are discussed as shown below :

```
. poisson λ01Δt1 LDLsp2 HOMAsp1 sysPS2 c.LDLsp2#c.HOMAsp1 c.LDLsp2#c.sysPS2 c.sysPS2#c.HOMAsp1, vce(robust) cformat(%9.3f) pformat(%5.3f
> ) sformat(%8.3f)

Iteration 0:   log pseudolikelihood = -112.93301
Iteration 1:   log pseudolikelihood = -110.47099
Iteration 2:   log pseudolikelihood = -110.43006
Iteration 3:   log pseudolikelihood = -110.43004
Iteration 4:   log pseudolikelihood = -110.43004

Poisson regression                              Number of obs     =        150
                                                Wald chi2(6)      =     535.34
                                                Prob > chi2       =     0.0000
Log pseudolikelihood = -110.43004               Pseudo R2         =     0.3552
```

λ01Δt1	Coef.	Robust Std. Err.	z	P>|z|	[95% Conf.	Interval]
LDLsp2	0.523	0.243	2.149	0.032	0.046	1.000
HOMAsp1	4.096	0.328	12.470	0.000	3.452	4.740
sysPS2	-0.628	0.347	-1.809	0.070	-1.308	0.052
c.LDLsp2#c.HOMAsp1	-0.179	0.070	-2.540	0.011	-0.317	-0.041
c.LDLsp2#c.sysPS2	0.003	0.000	8.144	0.000	0.002	0.003
c.sysPS2#c.HOMAsp1	0.151	0.098	1.547	0.122	-0.040	0.342
_cons	-9.510	0.725	-13.122	0.000	-10.930	-8.089

The above Stata command is used for regression of the counts of transition from state 0 to state 1 on the transformed predictors using robust standard error, the same command is used with the addition of irr to estimate the incidence rate ratio for this transition as shown below :

```
. poisson λ01Δt1 LDLsp2 HOMAsp1 sysPS2 c.LDLsp2#c.HOMAsp1 c.LDLsp2#c.sysPS2 c.sysPS2#c.HOMAsp1, vce(robust) irr cformat(%9.3f) pformat(%
> 5.3f) sformat(%8.3f)

Iteration 0:   log pseudolikelihood = -112.93301
Iteration 1:   log pseudolikelihood = -110.47099
Iteration 2:   log pseudolikelihood = -110.43006
Iteration 3:   log pseudolikelihood = -110.43004
Iteration 4:   log pseudolikelihood = -110.43004

Poisson regression                              Number of obs     =        150
                                                Wald chi2(6)      =     535.34
                                                Prob > chi2       =     0.0000
Log pseudolikelihood = -110.43004               Pseudo R2         =     0.3552
```

λ01Δt1	IRR	Robust Std. Err.	z	P>|z|	[95% Conf.	Interval]
LDLsp2	1.687	0.411	2.149	0.032	1.047	2.718
HOMAsp1	60.097	19.739	12.470	0.000	31.569	114.403
sysPS2	0.534	0.185	-1.809	0.070	0.270	1.054
c.LDLsp2#c.HOMAsp1	0.836	0.059	-2.540	0.011	0.728	0.960
c.LDLsp2#c.sysPS2	1.003	0.000	8.144	0.000	1.002	1.003
c.sysPS2#c.HOMAsp1	1.163	0.113	1.547	0.122	0.960	1.408
_cons	0.000	0.000	-13.122	0.000	0.000	0.000

The above results shows that the expected increase in log count for one-unit increase in transformed LDL cholesterol is (0.523), which is not highly statistically significant (P=0.032), and for one-unit increase in transformed HOMA is (4.096), which is highly statistically significant (P=0.000), as both are considered risk factors for NAFLD to progress from F0 to F1. The expected decrease in log count for one-unit increase in transformed systolic blood pressure is (0.628) which is not statistically significant (P=0.07). For every unit increase in transformed LDL, the incident rate ratio is increased (increase in transition counts) by 68.7%; while, for transformed HOMA, it is increased by 5909.7%, with 95% confidence that this increase is between 3056.9% and 11340.3%. The expected decrease in log count for one unit increase in interaction between the transformed LDL and transformed HOMA is (0.179) with high statistical significance (P=0.11), in other word, the rise in one predictor variable decreases the rising effect of the other on the response variable (expected log count) but not reverse it. And for every unit increase in this interaction, the incident rate ratio is decreased (i.e. decrease in transition counts) by 16.4%, with 95% confidence, that this decrease lies between 4% and 27.2%. While, the expected increase in log count for one unit increase in interaction between the transformed systolic blood pressure and transformed LDL is (0.003) with high statistical significance (P=0.000), and this increase in log count for one unit increase in interaction between transformed systolic blood pressure and transformed HOMA is (0.151) which is not statistically significant (P=0.122), in other word, the rise in one predictor variable increases the rising effect of the other on the response variable (expected log count).However, for every unit increase in the first interaction, the incident rate ratio is only increased (i.e. increase in transition counts) by 0.3%, with 95% confidence, that this increase lies between 0.2% and 0.31% , while for the second interaction; the IRR is increased by 16.3%.

To assess the fitness of the model, the output results in the Stata revealed the following goodness of fit, the AIC, and the BIC as shown below :

```
. estat gof

        Deviance goodness-of-fit =  27.55006
        Prob > chi2(143)         =    1.0000

        Pearson goodness-of-fit  =  24.45824
        Prob > chi2(143)         =    1.0000

. estat ic

Akaike's information criterion and Bayesian information criterion
```

Model	Obs	ll(null)	ll(model)	df	AIC	BIC
.	150	-171.2729	-110.43	7	234.8601	255.9345

Poisson model fits the data as goodness of fit is not statistically significant (P=1), and when compared to null model as shown in the output results of stata below, there is marked decreased in the deviance goodness of fit. Also the AIC and BIC are less than their values in the null model, which signifies the improvement in the full model. In addition there is increased in the pseudo R^2 indicating the ability of the model to predict the outcome better than the null model. The output results of the null model are shown below :

```
. poisson λ01Δt1, vce(robust) cformat(%9.3f) pformat(%5.3f) sformat(%8.3f)

Iteration 0:   log pseudolikelihood = -171.27294
Iteration 1:   log pseudolikelihood = -171.27294

Poisson regression                              Number of obs     =        150
                                                Wald chi2(0)      =          .
                                                Prob > chi2       =          .
Log pseudolikelihood = -171.27294               Pseudo R2         =     0.0000

------------------------------------------------------------------------------
             |               Robust
      λ01Δt1 |      Coef.   Std. Err.      z    P>|z|     [95% Conf. Interval]
-------------+----------------------------------------------------------------
       _cons |    -0.223      0.083    -2.696   0.007      -0.385      -0.061
------------------------------------------------------------------------------

. estat gof

        Deviance goodness-of-fit =  149.2359
        Prob > chi2(149)         =    0.4792

        Pearson goodness-of-fit  =     122.5
        Prob > chi2(149)         =    0.9449

. estat ic

Akaike's information criterion and Bayesian information criterion

-----------------------------------------------------------------------------
       Model |        Obs  ll(null)  ll(model)      df         AIC        BIC
-------------+---------------------------------------------------------------
           . |        150 -171.2729 -171.2729       1    344.5459   347.5565
-----------------------------------------------------------------------------
```

The first command in the below output results obtained from Stata is used to predict the $\ln \lambda_{01} = x_i' B$ and the second command is used to estimate the $E[y_i|x_i] = \lambda_{01} = e^{x_i' B}$, then rounding the previous result for the appropriate integer to obtain the estimated count of transition from state 0 to state 1. The forth command is used to obtain the frequency for each count made by the patients in the whole period of the study. The estimated number of transitions made in the person-year interval is 120 transitions and it is equals the observed counts.

```
. predict est01,xb

. gen est01count=exp(est01)

. gen est01countround=round( est01count )

. tab est01countround

est01countr |
      ound |      Freq.     Percent        Cum.
-----------+-----------------------------------
          0 |         75       50.00       50.00
          1 |         34       22.67       72.67
          2 |         37       24.67       97.33
          3 |          4        2.67      100.00
-----------+-----------------------------------
      Total |        150      100.00
```

The same procedure is implied for other transition counts and the results are illustrated in (the supplementary materials), with the associated discussion .

The comparisons between the distribution of the response rates and the estimated rates is illustrated in table (11) (See supplementary materials).

As the estimated rates approximately equal the observed rates obtained by CTMC especially when using the initial rates calculated as $\theta_0 = \frac{n_{ijr}}{n_{i+}}$ where the n_{ijr} is the transition counts from state i to state j and the n_{i+} is the total marginal transition counts out of this state i , as verified by the author; Iman Attia in previous 2 papers, and assuming that the marginal counts are the same, so the estimated Q transition rate matrix according to the estimated counts obtained by fitting Poisson regression is:

$$Q = \begin{bmatrix} -.059 & .059 & 0 & 0 & 0 \\ .029 & -.080 & .051 & 0 & 0 \\ .015 & .033 & -.093 & .045 & 0 \\ 0 & .108 & .158 & -.409 & .167 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix} \; where$$

$\lambda_{01} = \frac{120}{2050} = .059$, $\lambda_{12} = \frac{64}{1247} = .051$, $\lambda_{23} = \frac{35}{783} = .045$, $\lambda_{34} = \frac{20}{120} = .167$

$\mu_{10} = \frac{36}{1247} = .029$, $\mu_{21} = \frac{26}{783} = .033$, $\mu_{32} = \frac{19}{120} = .158$, $\mu_{20} = \frac{12}{783} = .015$, $\mu_{31} = \frac{13}{120} = .108$

Probability transition matrix is obtained from exponentiating this Q matrix after 1 year:

$$P(t=1) = \begin{bmatrix} .9435 & .0551 & .0014 & 0 & 0 \\ .0274 & .9247 & .0469 & .0009 & .0001 \\ .0144 & .0327 & .9149 & .0348 & .0032 \\ .0023 & .0863 & .1245 & .6512 & .1357 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$$

To calculate goodness of fit for multistate model used in this model, it is like the procedure used in contingency table, and it is calculated in each interval then sum up:

Step 1 : $H_0 = \; future\ state\ does\ not\ depend\ on\ the\ current\ state$

$H_1 = \; future\ state\ does\ \ depend\ on\ the\ current\ state.$

Step 2: calculate the $p_{ij}(\Delta t = 1) = \begin{bmatrix} .9435 & .0551 & .0014 & 0 & 0 \\ .0274 & .9247 & .0469 & .0009 & .0001 \\ .0144 & .0327 & .9149 & .0348 & .0032 \\ .0023 & .0863 & .1245 & .6512 & .1357 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix}$

Using exponentiation of the estimated Q matrix

Step3: calculate the expected counts in this interval by multiplying each row in the probability matrix with the corresponding total marginal counts in the observed transition counts matrix in the same interval to get the expected counts as shown below :

	State 0	State 1	State 2	State 3	State 4	Total
State 0	1934.175	112.955	2.87	0	0	2050
State 1	34.1678	1153.101	58.4843	1.1223	0.1247	1247
State 2	11.2752	25.6041	716.3667	27.2484	2.5056	783
State 3	.276	10.356	14.94	78.144	16.284	120
State 4	0	0	0	0	0	0

Step 4: apply $\sum_{i=1}^{5}\sum_{j=1}^{5}\frac{(O_{ij}-E_{ij})^2}{E_{ij}} = 1140.097 \sim \chi^2_{(5-1)(5-1)(.05)}$.

So from the above results the null hypothesis is rejected while the alternative hypothesis is accepted and the model fits the data that is to mean the future state depends on the current state with the estimated transition rates and probability matrices as obtained.

Of those patients starting at F0 ,only 5.51% will move to F1 in one year, this declines to 4.69% of patients starting at F1 moving to F2 ,while 3.48% of patients starting at F2 will move to F3 ; however, 13.57% of patients starting in F3 will move to F4, and this high percentage of patients moving towards advanced fibrosis may be due to the fact that advanced fibrosis is considered to be F3 and F4 and once the patient reaches F3 , his chance to progress to F4 is higher than being in any starting stage considered less advanced fibrosis including F0 to F2 (by definition), and this is obvious as shown by incidence rate ratio of this transition being the highest (5.237e+6) . It is shown that progression from F0 to F1 and from F1 to F2 is approximately equal, while transition from F2 to F3 is less and this may be to more aggressive intervention taken by the patients to hinder the progression of fibrosis by applying more intensive lifestyle modifications, but once the patient reaches stage F3 the progression to F4 is by

far the most among the forward transitions. There are 2.74% of patients starting at F1 will move to F0 while this percentage decreases to 1.44% if starting at F2, and it is even less if starting at F3 (only .23 % of patients can achieve this task); hence it is more feasible to move from F1 to F0 than to move from F2 to F0 than to move from F3 to F0; that is to mean, the more advanced the stage of fibrosis the patient experiences, the less likely movement to F0 he affords to do. There is a paradox if the starting stage is F2 or F3 to F1. The movement to F1 is more obvious if the patient is in F3 (8.63% of patients move to F1) than if he is in F2 (3.27 % of patients move to F1); therefore, the more advanced fibrosis stage the patient recognizes , the more likely movement to F1 he can do, and may be this is due to the extensive lifestyle modification he performs to achieve less degree of fibrosis, but it remains a little bit difficult to reach F0 (only .23 % of patient can move from F3 to F0). It is also noted that 2.74% of patients move from F1 to F0 , 3.27% of patients move from F2 to F1 while 12.45% of patients move from F3 to F2 ; in other words the more advanced the fibrosis stage is, the more likely the movement to the immediately previous stage is. Moreover if the starting stage is F3, then 13.57% of these patients move to F4, a little bit higher than moving to F2 (12.45% of the patients); whereas, movement to F1 and F0 declines (8.63% of the patients and .23% of the patients respectively, approximately movement to F0 is 2.66% that to F1). Of those patients starting in F2, 3.48% move to F3, a little bit more than moving to F1 (3.27 % of patients); nevertheless, movement to F0 is almost 44% that to F1 (1.44% of the patients move to F0).

Mean time spent by the patient in state 0 is approximately 17 years that declines to 12 years and 6 months spent in state 1, which further declines to approximately 10 years and 9 months spent in state 2, and ultimately reaching 2 years and 3.7 months spent in state 3. It is shown that, there is decrease in time spent in each stage as the disease process evolves over time. This huge rapid decline in time spent in state 3 is due to advanced fibrosis induced by dead hepatocytes, especially if no treatment is introduced like: lifestyle modification ,risk factors treatment, as well as anti-inflammatory and anti-fibrotic drugs, and if so, it is a matter of time to reach state 4, which is irreversible stage of damaged liver cells that will soon manifest with reduction in liver cell functions, and may be to hepatocellular carcinoma, and eventually death, if not managed with liver transplantation.

3. Conclusions:

Insulin resistance is a key stone for triggering all these abnormalities, the more sensitive the body cells is to insulin, the less likely the complications of NALFD will develop. The effect of risk factors or covariates as a mainstay players, like: increased insulin resistance, hyperlipidemia with increased LDL-cholesterol, high systolic and diastolic blood pressure are thoroughly explained using the Poisson regression model combined with CTMC. As concluded from the hypothetical model that for every unit increase in the transformed HOMA, the incidence rate ratio for transition from state 0 to state 1 is increased by 5909.7% and this elevation is kept rising while moving forward from subsequent state to the immediately next state, that is to mean, for every unit increase in the transformed HOMA, the incidence rate ratio (IRR) for transition from state 1 to state 2 is increased by 24017.9%, while for the transition from state 2 to state 3, it is increased by 47931.8% , and for transition from state 3 to state 4 it is increased by 5237498.4%. This increment is almost always highly statistically significant. This is in comparison with transformed LDL, as for every unit increase in the transformed LDL, the IRR for transition from state 0 to state 1 is increased by 68.7%, while for the transition from state 1 to state 2, it is increased by 36.4% , and for transition from state 3 to state 4 it is increased by 57.1%. And it is only highly statistically significant for transition from state 3 to state 4. However the systolic blood pressure is almost highly statistically significant for the transition from state 2 to state 3 as obvious by for every unit increase in the transformed systolic pressure, the IRR for this transition to occur is increased by 1114.3%. Moreover, for every unit decrease in the transformed HOMA, the IRR for transition from state 1 to state 0 is increased by 1.1%, for transition from state 2 to state 1 it is increased by 3.7%, for transition from state 3 to state 2 it is increased by 0.5%, for transition from state 2 to state 0 it is increased by 6.6%, and for transition from state 3 to state 1 it is increased by 8.4%. This emphasizes that better control of insulin resistance helps the patient to reverse his condition. To sum up, the precipitating factors should be rigorously and extensively treated and controlled by life style modifications represented by dietary restriction of high calorie diet and sedentary life, thus the predisposed persons should consume healthy diets and regularly practicing physical exercises suitable for their medical conditions. The newly discovered drugs like anti-fibrotic drugs that treat the fibrotic changes in the liver are promising drugs and await further longitudinal studies, to reveal the most effective protocol, by which they are administered to the patients, for better control of the rate of progression of liver fibrosis. This control keeps the patient out of loss of liver functions, and subsequently away

from end stage liver disease, which necessitates liver transplantation with all its accompanying post transplantation complications.

4.Programs and Supplementary Materials

The above example is published with Stata data and the accompanied do file, as well as supplementary file on code ocean site with the following URL:
Codeocean.com/capsule/4752445/tree/v1

Abbreviations:
CC: compensated cirrhosis (stage 4),CTMC: continuous time Markov chains, DCC: de-compensated cirrhosis (stage 5),EM: extra-mortality (stage 9),HCC :hepato-cellular carcinoma (stage 8),LT: liver transplant(stage 6),NAFLD: non-alcoholic fatty liver disease, NAFLD-NO FB : nonalcoholic fatty liver disease with no fibrosis (stage 1),NASH: non-alcoholic steatohepatitis, NASH-NO FB : nonalcoholic steato-hepatitis with no fibrosis (stage 2), NASH-FB: nonalcoholic steato-hepatitis with fibrosis (stage 3),PLT : post liver transplant (stage 7),T2DM: type 2 diabetes mellitus.

Declarations:
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable
Availability of data and material
Not applicable. Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.
Competing interests
The author declares that I have no competing interests.
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Authors' contribution
I am the author who has carried the mathematical analysis as well as applying these mathematical statistical concepts on the hypothetical example.
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